Relations entre phénologie de la croissance souterraine et aérienne de noyers hybrides en systèmes agroforestiers tempérés

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To cite this version:

Awaz Mohamed. Relations entre phénologie de la croissance souterraine et aérienne de noyers hybrides en systèmes agroforestiers tempérés. Life Sciences [q-bio]. Université de Montpellier, 2016. English. NNT : . tel-02795726v1

HAL Id: tel-02795726
https://hal.inrae.fr/tel-02795726v1

Submitted on 5 Jun 2020 (v1), last revised 15 Jan 2018 (v2)

THÈSE
Pour obtenir le grade de
Docteur

Délivré par **Université de Montpellier**

Préparée au sein de l'école doctorale GAIA
Et de l'unité de recherche UMR AMAP

Spécialité : **Sciences Agronomiques**

Présentée par **Awaz Mohamed**

Relations entre phénologie de la croissance souterraine et aérienne de noyers hybrides en systèmes agroforestiers tempérés

Soutenue le **07 Décembre 2016** devant le jury composé de

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Remerciements

Soyons reconnaissants aux personnes qui nous donnent du bonheur;

elles sont les charmants jardiniers par qui nos âmes sont fleuries.

Marcel Proust

Grand merci aux personnes qui ont cru en moi et qui m'ont permis d'arriver au bout de cette thèse.

Je tiens tout d'abord à remercier les membres de jury pour avoir accepté d'évaluer le travail de cette thèse. Merci donc aux rapporteurs, **Michel-Pierre Faucon** et **Laurent Saint-André,** Ainsi qu'aux examinatrices **Claude Plassard** et **Camille Beral.**

Mes premiers mots vont ensuite à ma directrice de thèse **Alexia Stokes.** Merci de m'avoir montré les clés du succès dont avoir confiance en soi et en ses capacités, croire en soi et toujours tenter de se dépasser. Du fond de cœur, je te remercie pour ta patience, ta gentillesse, ta simplicité, ta bonne humeur communicative et ton aide dans les moments les plus difficiles.

Je tiens à remercier très chaleureusement mon Co-Encadrant **Yogan Monnier** pour avoir Co-encadré cette thèse. Merci donc pour ta gentillesse, ta patience, tes précieux conseils et ta faculté de me motiver et positiver pendant les périodes un peu chaudes.

J'adresse ma gratitude au Campus France et l'institut kurde à Paris pour m'avoir donné cette chance d'effectuer cette thèse en France. Mention spéciale à **Kendal Nezan**, **Joyce Blau** et **Avin Babataheri**.

Un grand merci à **Pierre Couteron** et à **Thierry Fourcaud** pour m'avoir accueilli dans les locaux du laboratoire UMR AMAP. Un grand merci également aux agriculteurs **M. Queuille and M. Bécue** pour m'avoir laissé travailler dans leurs parcelles.

Je tiens à remercier l'ensemble des techniciens : **François Pailler**, **Merlin Ramel** et **Stéphane Fourtier** sans qui je ne pourrais jamais arriver à réaliser ce travail. Merci donc pour m'avoir enseigné les différents protocoles et m'avoir accompagné sur le terrain. C'était un grand plaisir de travailler avec vous.

Une pensée particulièrement aux assistantes **Noémie Cauquil**, **Nathalie Hodebert** et **Yannick Brohard** pour leur aide dans mes démarches administratives lors de ces trois années.

Grand merci à **Guillaume Lobet** de m'avoir aidé dans l'utilisation du logiciel de l'analyse d'images SmartRoot. Merci également à **Jean-Luc Maeght**, **Christophe Jourdan** et **Sylvie Sabatier** pour leurs aides, les discussions et les conseils qu'ils m'ont fournis et qui m'ont beaucoup apporté.

Un grand merci à l'équipe technique, à l'UMR SYSTEM, **Lydie Dufour**, **Jeff Bourdoncle** et **Alain Sellier** pour leurs aides sur le terrain à Restinclières.

Mes remerciements vont aussi pour mes premiers pas dans l'encadrement de stages, mes deux petits stagiaires **Antoine Tournier** et **Mathilde Millan.** Merci donc pour leurs aides en récupération des données sur le terrain et l'analyse des milliers d'images.

Je remercie bien tous les membres de l'équipe EASE (Ecologie appliquée et services écosystémiques) pour l'ambiance sympathique et les gâteaux délicieux durant les nombreuses discussions que j'ai pu avoir avec chacun de vous et qui m'ont beaucoup apporté. Je remercie donc toutes ces personnes. Je tiens à remercier particulièrement **Mao Zhun** pour m'avoir accompagné dans l'analyse des données**, Luis Merino-Martin** et **Lorenzo ROSSI** pour m’avoir aidé sur le terrain. Une mention spéciale à **Amandine Erktan** pour son aide et ses discussions sympas et ses conseils qui m’ont accompagné pendant de ces trois années en partageant le même bureau. Du même, une mention spéciale à **Jérôme Nespoulous**, Merci de m’avoir aidé et supporté scientifiquement et personnellement pendant ces années. J'ai beaucoup apprécié de travailler à tes côtés et c’était génial tout le courage qu’on s’est donné, tous les bons repas qu’on a partagé et tous les bons moments qu’on a passés ensemble au labo ou hors labo. Merci également à ton vélo qui nous a transportés pendant ces années. Grâce à toi, j’ai découvert une nouvelle culture et pleine des activités. SPAS donc du fond de mon cœur pour tout.

Un énorme MERCI pour des amis tout simplement géniaux de prés et de loin! Qu'aurais-je fait si vous n'étiez pas là? Surement pas grande chose ! Mention spéciale pour les deux anges **Pamela El-Hajj** et **Nathalie Al-Makdessi** qui m’ont supporté et accompagné pendant ces années avec un cœur d’Or. Merci du fond de cœur de m’avoir partagé la vie et d’être toujours à mes côtés même dans les moments les plus difficiles. Vous n’êtes pas mes sœurs de sang mais vous êtes quand même mes sœurs de cœur. Je vous aime mes sœurs chéries.

Merci également à **Darin Genty, Nicolas Solignac** et **Alain Ibrahim** pour m’avoir soutenu dans les périodes un peu chaudes.

En fin, les mots les plus simples étant les plus forts, j’adresse toute mon affection à ma famille, et en particulier à mes parents qui m’ont toujours encouragé et supporté. Malgré mon éloignement depuis de nombreuses années, leur intelligence, leur confiance, leur tendresse, leur amour me portent et me guident tous les jours. Spas pour tout. Je vous aime. Merci également à mes frères et mes sœurs pour leurs encouragements et support pendant ces trois années. Merci donc à **Jwana**, **Suha**, **Ahmad**, **Omar** et **Mustafa**. Un grand merci à mon oncle **Ahmad Abdulkarim** grâce à qui je suis arrivée à ce que je suis aujourd’hui. Merci donc pour tout le soutien que tu m’as donné pour arriver à réaliser ce travail.

To all my friends in Syria who scattered around the country, thank you for your thoughts, well-wishes/prayers, phone calls, emails, texts, advice, and being there whenever I needed a friend. Especially, I must express my very profound gratitude to my wonderful friend **Dima**. Though miles lies between us, we're never far apart, for friendship doesn't count the miles, it's measured by the heart. Thanks for always being there for me. I love you so much my darling sister and hope to see you again one day, once this war stop.

Finally, I would like to tell all Syrians who suffer because of this present war that none of you is forgotten! I send love to you! Keep hope alive!

My only hope is for peace in Syria soon.

Funding

Funding for a PhD. bursary was provided by Campus France and the Kurdish Institute, France (AM) and fieldwork was funded by the FOARDAPT project, INRA metaprogram AAFCC (Adaptation of Agriculture and Forests to Climate Change), France.

Résumé

L’étude de la phénologie des plantes est primordiale pour comprendre leur réponse aux changements globaux. Alors que de nombreuses études ont été consacrées à la phénologie aérienne, la phénologie des parties sous terraines est encore peu connue, du fait des difficultés d’observation. La dynamique racinaire est pourtant considérée comme jouant un rôle clef dans le cycle et la séquestration du carbone dans le sol. Il est donc aujourd’hui important de mieux comprendre les contrôles climatiques de cette dynamique racinaire sur plusieurs années en conditions non-contrôlées. Notre premier objectif ici était d’évaluer différentes techniques d’observation de la croissance racinaire à moyen terme sur le terrain. Le modèle choisi pour cela est le noyer hybride (*Juglans* L.), en système agroforestier. L’utilisation du scan incorporé au smartphone s’est avéré être le meilleur compromis pour l’acquisition d’image à partir de rhizotrons. En cas de nécessité d’automatisation de la prise d’images, la caméra automatique constitue la meilleur alternative (jusqu’à 4 mois d’autonomie). Notre deuxième objectif était de déterminer en milieu naturel le rôle des variables climatiques sur la dynamique racinaire. Nous avons (i) testé l’hypothèse de la synchronicité des phénologies aériennes et racinaires de la plante, (ii) évalué la dynamique racinaire dans des environnements contrastés (climats méditerranéen, océanique, continental), et (iii) cherché à comprendre si cette réponse aux facteurs climatiques était conditionnée par la typologie racinaire. Les résultats ont montré l’indépendance des phénologies aériennes et racinaires, mais la synchronisation des croissances racinaires et radiale du tronc. Le principal facteur influençant la dynamique racinaire est la température du sol, et à moindre mesure l’humidité du sol avec des effets contrastés selon le climat. Les réponses des dynamiques racinaires aux variables climatiques restent propres à chaque site, avec un fort impact de l’ordre topologique. Un troisième objectif était d’étudier la phénologie des racines d’horizons ‘profond’ (2-4m), et leur synchronicité vis-à-vis des autres parties de l’arbre. Nous avons montré que la production de racines profondes se faisait de manière asynchrone des parties aériennes et racinaires superficielles, quasi indépendamment des fluctuations climatiques, avec des pics de croissance jusqu’en période hivernale. Ces résultats permettront non seulement une meilleure compréhension du rôle joué par les systèmes racinaires sur le cycle du carbone, mais aussi l’amélioration des modèles écophysiologiques. De futures études sont néanmoins nécessaires pour renforcer les connaissances acquises dans ce projet, sur la compréhension des déterminants de l’initiation, de la croissance, et de la longévité racinaire des arbres, notamment via la création de bases de données et l’utilisation de méta-analyses.

Summary

The study of phenology is primordial to understand tree response to climate change. Although many studies have examined shoot phenology, the difficulties in observing root system growth have resulted in a poor understanding of root phenology. As root system dynamics are considered as playing a major role in carbon cycling and sequestration, it is necessary to overcome methodological difficulties, so that root demography can be studied in the field and over several years. Our first objective was therefore to develop and evaluate methods for studying root system growth in the field. Studying mature hybrid walnut (*Juglans* L.), growing in agroforests, We showed that smartphone scanners are the best adapted tool for acquiring high quality images of tree roots growing in field rhizotrons. However, time-lapse cameras were good alternatives when a fully automated method was required (up to 4 months autonomy). Our second objective was to determine the main drivers of walnut hybrid root growth in field conditions. In particular, (i) We hypothesized that shoot and root phenologies were asynchronous, (ii) We evaluated the effect of environmental factors on root growth along a latitudinal gradient comprising three climates (Mediterranean, oceanic and continental) and (iii) We determined how phenology and environmental factors influenced root dynamics depending on root morphology and topology. Results show that flushes of fine root growth are not synchronized with budburst and leaf expansion, but are synchronized with stem and coarse root radial growth. Soil temperature was overall the main driver of root growth, and to a lesser extent, soil humidity, but which had contrasting effects on root growth. Root topological order had a major influence on root response to environmental variables, reflected in root elongation, production and longevity. Our third objective was to put more focus on the relationships between deep fine roots and shoot phenology of walnut trees (*Juglans negra* x *Juglans regia* L.) growing in Mediterranean alley-cropping system. Results show that fine root phenology was asynchronous with leaf phenology in all soil depths. Whereas, roots at the two upper soil layers only were synchronous with radial growth of both stem and coarse roots. Roots at deeper soil layers were independent of the climatic variables and flushed later than those at shallow soil layers reaching to maximal rate of growth in autumn and winter. Results will be highly useful not only for a better understanding of the role that root systems play in the carbon cycle, but also for tree ecophysiological models. Future studies should expand the knowledge gained here into a global understanding of the drivers of tree root initiation, growth and longevity, through the creation of databases and the use of meta-analyses.

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Chapter I: General Introduction

1. What are fine roots?

Fine roots are traditionally thought of as those roots < 2 mm in diameter (Pregitzer et al 2002) (Wang et al 2007). Fine roots link plant metabolism to soil nutrient cycles and are ephemeral and frequently replaced. However, recent studies have defined fine roots not by diameter class but by their function and behavior and their position in the branching root system (Majdi et al 2005). Fine roots have been classified into two distinct classes (i) absorptive roots which represent the most distal roots and are implicated in the acquisition and uptake of soil resources. (ii) roots with a transport function that occur higher up in the branching order and also possess some capacity for storage (McCormack et al 2015).

2. Why study fine roots?

Roots play a key role in the cycling and the allocation of carbon (C) and nutrients (Clark et al 2013) (Gill & Jackson 2000). Globally, 20%–40% of the forest biomass is made up of roots (Litton et al 2007). A significant amount of C assimilated by plants through photosynthesis is transferred and allocated to roots (McCormack et al 2015) and this amount exceeds the amount allocated to aboveground components (Eissenstat & Yanai 1997) (Hertel et al 2009). Root C contributes to the total belowground C pool by about 42% (Brunner & Godbold 2007). Quantifying the pattern of fine root dynamics in response to changes in atmospheric CO_2 concentrations, temperatures, precipitation, or nitrogen deposition, is crucial to the understanding of ecosystem structure and function and in predicting how ecosystems respond to climate variability (Norby & Jackson 2000).

3. Why Agroforestry?

3.1. Definition

In simple terms, agroforestry is a land use management system in which woody perennials (tree, shrub, etc.) are associated with herbaceous plants (agricultural crops, pastures) and/or livestock in a spatial arrangement, a rotation or both (Somarriba 1992).

3.2 Agroforestry and ecosystem services

Ecosystem services are the benefits that humans receive from his ecosystem including (i) provisioning services such as food and water; regulating services such as regulation of floods, drought, land degradation, and disease; (ii) supporting services such as soil formation and nutrient cycling; and (iii) cultural services such as recreational, spiritual, and religious and other nonmaterial benefits (Millennium Ecosystem Assessment (MA), 2005). The demand for ecosystem services is now so great due to the increase of human demands on ecosystems and the decrease of global biodiversity at unprecedented rates (TEEB Synthesis, 2010). Current studies estimate 3 billion more people by 2050, which implies a formidable increase of human consumption of resources, as well as escalating impacts on ecosystems and the services provided. For example, meeting the needs for a growing world population for food supply could be increased by converting a forest to agriculture but in so doing we decrease the supply of services that may be of equal or greater importance, such as clean water, timber, ecotourism destinations, or flood regulation and drought control.

Agroforestry optimizes all three ecological, economic and social benefits created by the interactions between the trees and crops and livestock (Franzen & Mulder 2007). These interactions protect the environment and offer a number of ecosystem services (Jose 2009, Newaj et al 2016) such as biodiversity conservation (Harvey et al 2008) (Schroth & Harvey 2007), soil enrichment (Schroth & Sinclair 2003), water quality enhancement (Anderson et al 2009), carbon sequestration (Dixon et al 1994) (Kirby & Potvin 2007), decreasing soil erosion and land degradation (Ramachandran Nair et al 2009) (Garrett et al 2009) (Garrity 2004). Moreover, agroforestry systems were mentioned by their important roles in adaptation and mitigation to climate change. For example, trees provide protection to crops and animals during warming (Ramachandran Nair et al 2009) and mitigate the greenhouse effect by sequestering a large quantity of carbon in their tissues and in soil especially the root systems (Verchot et al 2007).

All these ecosystem services depend on the type of agroforestry practices. Agroforestry practices are classified into five main practices (Alao & Shuaibu 2013, Jose 2009):

(i) Forest farming: cultivation of a valued crop under the protection of a forest canopy, modified to provide the correct level of shade providing short-term income while high quality trees are grown for wood products.

(ii) Silvo-pasture: combining trees, forage and livestock by planting (perennial grasses or legume mixes between rows of widely spaced trees for livestock pasture, and hence decreasing the feeding costs for livestock and improving animal health.

(iii) Alley cropping: planting rows of trees at wide spacing while a companion crop (wheat, corn, pea, or soybeans) grows in the alleyways between the rows, which improves crop production by protecting and conserving benefits to crops.

(iv) Riparian forest and upland buffers: combining trees, shrubs, forbs and grasses, including native plants. This combination helps to enhance filtration of nutrients from surface run-off and shallow ground water and thus protects the water quality of lakes, controlling soil erosion and providing food and cover to wildlife.

(v) Wind breaks: system managed as part of crop or livestock for protecting a variety of wind-sensitive crops. Such a system enhances production, controls wind erosion, increases bee pollination, and reduces spray drift of pesticides.

3.3 The role of roots in agroforestry systems

Tree roots can have positive effects on associated soil and crops in agroforest systems e.g., soil carbon enrichment through root turnover, increasing water infiltration and retention in the soil profile by increasing soil porosity and reduced runoff (Gyssels et al 2005) (Newaj et al 2016). Tree roots are able to expand over a large volume of soil for water and nutrient uptake and in doing so, increase soil aggregate stability and thus improve soil fertility (Buck et al 1998). The facilitative effects of trees and associated crops include deep-rooting trees that are able to perform hydraulic redistribution and take up a higher volume of soil water and nutrients (Udawatta et al 2002) which are less accessible to shallow-rooted crops, leading to a complementarity of the use of soil resources (Schroth 1998). However, knowledge about below ground processes in such systems is scanty.

4. Root methodological problems

Despite the fundamental role of fine roots in ecosystem functions and nutrient and carbon cycling, knowledge about events in below ground processes is still limited due to the accessibility of root systems (Maeght et al 2015, Vogt et al 1998). Methods of monitoring root growth and specific root characteristics have ranged widely in technique and or equipment needed. These methods can be grouped into indirect (e.g. use of empirical models (Kurz et al 1996), estimations of nitrogen (N) budget and C budget (Hendricks et al 2006) and direct techniques (Hendricks et al 2006, Vogt et al 1998), both of which have advantages and drawbacks.

Direct methods are used to monitor roots in their natural states e.g., (i) destructive techniques such as soil core (Box & Ramsuer 1993), sequential soil coring (Vogt et al 1998), in-growth cores (Makkonen & Helmisaari 1999, Vogt et al 1998), monoliths (Majdi et al 1992)[11] and (ii) nondestructive techniques such as isotope quantification (Strand et al 2008) ,‘root windows’ or rhizotrons (Bates 1937, Burke & Raynal 1994, Mao et al 2013a, Reich et al 1980) and minirhizotrons (Germon et al 2016, Steinaker et al 2010, West et al 2004). The choice of the best root study method depends on the research aim. Although the true answer for the ideal method is never known because each method has potential biases (Van Do et al 2016) (Yuan & Chen 2012), rhizotrons and minirhizotrons are commonly considered as the most efficient approaches providing detailed information about temporal and spatial changes in root activity (Eissenstat & Caldwell 1988, Hendrick & Pregitzer 1996, Majdi 1996) (Taylor et al 1991). However, the drawbacks of these techniques are related to the cost of installation and disturbance in soil hydrology and physics, which would affect the amount of root production (Hendricks et al 2006, Taylor et al 1991). A number of studies using minirhizotrons have been performed (Steinaker et al 2010, Tanner et al 2006). However, observations of root growth in minirhizotrons by recording root images with digital cameras (Fukuzawa et al 2013)[29] or rotating scanners (CID, Inc, WA, USA) (Germon et al 2016) is very expensive and only a small part of root systems can be observed. Rhizotrons are a cost-effective, nondestructive way to monitor root growth in situ offering a larger measurable area and creating better contact between roots and soil. In addition, the advantage of rhizotrons over minirhizotrons, is that a variety of techniques exist for quantifying root growth in the field. Root systems can be measured by tracing onto a transparent plastic sheet (Mao et al 2013a) or scanning with different scanners models (e.g. flatbed, handheld). Image analysis softwares are progressively emerging and have developed to facilitate analyzing procedures (Himmelbauer et al 2004). Analyzing procedures in such softwares depends principally on image quality. Scanners have often been considered as the most useful tool for obtaining high quality images (Dannoura et al 2008), but necessitate the use of a power supply in the field and are not yet fully automated. However, a detailed comparison of the different types of scanners available has not yet been performed, especially with regard to the scanners now available as digital applications on smartphones and tablets. Such a comparative study would be highly useful. Moreover, there are no products currently available for automatically capturing root images *in situ* when using rhizotrons. A fully automated method for measuring root growth in the field would permit studies of growth in poorly accessible areas or with a poor power supply, as well as detailed measurements of e.g. effects of the circadian clock on root growth in situ (Halter et al 1996).

5. What drives fine root dynamics?

It is widely acknowledged that fine root dynamics, defined as elongation rate (Germon et al 2016, Jourdan et al 2008), production and mortality (Hendrick & Pregitzer 1993b) (Mao et al 2013b) (McCormack et al 2014), turnover (Anderson et al 2003), survivorship (Anderson et al 2003) (Kern et al 2004) and, senescence (Huck et al 1987), could be strongly driven by both endogenous and exogenous factors, which include plant age, species, cultivars, soil temperature (Tanner et al 2006) (Steinaker & Wilson 2008) (Steinaker et al 2010) (Coll et al 2012), moisture (Metcalfe et al 2008, Misson et al 2006) (Block et al 2006), hormones (McAdam et al 2016), photosynthate avaibility and nutrient availability (Sloan et al 2016) (Tierney & Fahey 2002). In regions with distinct seasons, it is widely recognized that root growth is often correlated with the variation in soil temperature (McCormack et al 2014) (Mao et al 2013a). Thus, increased temperatures should reduce root life span (Chen & Brassard 2013) and promote higher rates of root production (Radville et al 2016a, Wan et al 2004). For example, Misra (1999), found that root elongation increased with an increase in temperature and decreased sharply when temperature decreased. However, (Joslin et al 2001) failed to find a correlation between root elongation intensity and soil temperature and concluded that soil water potential played a major role in driving root elongation intensity. Tierney et al (2003) (Tierney & Fahey 2002)found that monthly fine root production was strongly associated with mean monthly air temperature and not with soil moisture nor nutrient availability. Many other studies have not found a correlation with abiotic factors and root production (Hendrick & Pregitzer 1993a, Hendrick & Pregitzer 1993b, Hendrick & Pregitzer 1996) (Joslin & Wolfe 1998, Joslin et al 2000) and suggest that root morphological traits (Mao et al 2013c) (Kern et al 2004) and endogenous factors such as hormones[34], photo-assimilate transport and photosynthate availability (Sloan et al 2016) (Tierney & Fahey 2002) (Joslin et al 2000) are the main drivers of growth. Furthermore, various plant species react differently to various climatic factors, even in different phenological phases within the same species (Wielgolaski 2003). For example, Mapelli et al (2013) (Mapelli et al 1995) showed that walnut seedlings have a low resistance to water stress and are sensitive to waterlogging both between and within cultivars.

5.1. Different roots, different roles

The root system architecture of plants varies hugely between species and variations are also found within species (Fitter 2002) (Cannon 1949) (Nibau et al 2008). Studies of root system architecture take into account the morphology and topology of a root system over time. Root size is extremely important in evaluating a plant’s ability to survive in stressful or poor soils, because roots differing in size also differ in their structural traits (Majdi et al 2005, Mao et al 2013a). Tree roots are generally classified by diameter into fine roots and coarse roots. Coarse roots are larger in size, more lignified and have longer survivorship than fine roots, which possess an absorptive function (Stokes et al 2009). Although the morphology of a root system greatly influences its role in carbon and nutrient cycling, limited studies have been performed in the context of how climate variability could alter the growth and longevity of different roots sizes throughout the year.

5.1.1. Classification of roots by diameter

Root diameter is one of the main criteria taken into account in studies of root architecture (Jourdan & Rey 1997). Root diameter is not constant along the root and is modified with age and the local environment. Roots are classified by classes of diameter, fine roots and coarse roots. The threshold between the two classes varies highly among species and study objectives (Fogel 1983, Harris et al 1980) pointed out that there was no established convention defining the diameter range of fine roots. Thus, some consider that fine roots are of diameters less than 1 mm, 2 mm or even 5 mm in diameter (Wells & Eissenstat 2001) (Wells & Eissenstat 2001). Others studies named thick roots as those >10 mm in diameter. Different root diameter classes possess different roles, for example, Pollen-Bankhead et al. (2011) (Pollen-Bankhead et al 2011) highlighted that roots <5 mm in diameter played the most important role in reinforcing soil on a slope. Other studies reported that roots with small diameters lived for shorter times at shallow soil depths and cycled more rapidly than thicker or deeper roots (Kern et al 2004) (Germon et al 2016, Wells & Eissenstat 2001). Mao et al, (2013) found that root elongation diminished with decreasing class of diameter. However, knowledge about the response of roots in different topological orders with similar diameter classes to climate variability is scarce.

5.1.2. Classification of roots by topology orders

Root topology describes the physical connections between roots in the root system network, and is an important indicator to characterize root system architecture (Mao et al, 2013). Root topological order is considered an important trait, because it affects nutrient uptake (Fitter & Stickland 1992) and soil fixation (Fan & Chen 2010). Different topological orders of roots differ in their concentration of C and N (Pregitzer et al 1998) (Luke McCormack et al 2012) and react differently to the local environmental conditions with regard to the longevity of roots (Wells & Eissenstat 2001) (Tierney & Fahey 2002). Despite this, research on the relationship between root topology orders and abiotic factors is scanty.

6. The role of deep fine roots

The importance of fine roots in carbon and nutrient cycling in temperate deciduous forests is well documented (Hendrick & Pregitzer 1996). Most studies have focused on shallow roots (<1), consequently, little is known about deep root phenology and their roles in carbon and nutrient cycling (Maeght 2015). Tree deep roots play a significant role in hydraulic redistribution particularly in regions with drought summer (e.g., Mediterranean climate) (Udawatta et al 2002) by expanding over a large volume of soil, thus taking up higher volum of water and nutrients and improving in the same time soil aggregate stability and soil soil fertility (Buck et al 1998). Fine root phenology differs by depth in certain ecosystems and may be controlled by different factors (Radville et al 2016). Deeper soil layers assumed to be less sensitive to climatic changes than superficial soil layers because soil buffered against the rapid changes in air temperature (Du & Fang 2014, Radville et al 2016) resulting asynchrony with above ground phenology (e.g. when air temperature rise faster). We expect that climatic factors will not affect in the same way shallow and deep roots and that deeper root phenology may shift later than shallow root phenology.

7. Are above and below ground phenology in sync?

Climate models predict that an increase in atmospheric CO_2 concentration, precipitation and temperature could affect many biological phenomena and increase the frequency and magnitude of extreme weather events (IPCC, 2007) (Parry et al 2007). Predicting the response of ecosystems and vegetation to climate variations has become a major challenge for research (Casassa et al 2007). Recent studies have considered the changes in plant phenology as a very sensitive and observable indicator of plant responses to climate change (Radville et al 2016a). Most studies on plant phenology have focused on above ground phenology which is more accessible and is directly related to photosynthesis production (Du & Fang 2014) (Menzel

2003), while much less is known about root phenology (Steinaker and Wilson 2008). Root phenology can be separated into many events such as onset, peak and cessation, while leaf phenology can be separated into budburst and leaf senescence (Radville et al 2016a). However, more focus is needed on understanding the driver and the timing of each event of root growth.

Root and shoot phenology may be closely related because shoots are dependent on roots for soil resources and roots dependent on shoots for photosynthates (Steinaker et al 2010). Shoot and root phenology could be strongly controlled by different factors (biotic and abiotic) throughout the year (Radville et al 2016a, Radville et al 2016b). Temperature may be the most important abiotic factor controlling spring phenology in temperate climates (Radville et al 2016a, Wielgolaski 2003). Both budburst and root emergence are very sensitive to temperatures (Du & Fang 2014, Tierney & Fahey 2002). The timing of production can vary between roots and shoots (Blume-Werry et al 2016). Some studies showed that root growth peaks early in spring and was synchronous with shoot phenology (Misson et al 2006) (Germon et al 2016), while other studies demonstrated that root growth lagged behind shoot phenology. Shoots preceded roots in arctic, Mediterranean, boreal and temperate biomes (Abramoff & Finzi 2015) (Blume-Werry et al 2016) (Du & Fang 2014, Steinaker & Wilson 2008), but roots preceded shoots in the subtropical biome (Abramoff & Finzi 2015). However, simultaneous measurements of root and shoot phenology remain scarce (Steinaker & Wilson 2008) (Sloan et al 2016).

8. General hypotheses and objectives

In this thesis, we ask multiple questions:

What is the best technique for root image acquisition with regard to the quality of image, time and cost when using *in situ* rhizotrons? What drive fine root dynamics along a latitudinal gradient? Are the drivers of root growth and mortality the same? Are shoot and roots in sync? Do root morphological traits influence root survivorship? Are shoot and root growth under the same controls? Does fine root phenology differ by depth?

Our principal hypotheses are the following:

- Shoot and root are asynchronous regardless of either the climate or the soil depth.
- The drivers of root growth and root mortality are not the same between climates.
- Root diameter is linked to elongation rate and is altered over phenological periods.
- Root longevity is altered between root morphological traits among climates.
- Shoot and root growth are under different controls.
- The drivers of root growth are not the same between soil depths

Objectives:

- Quantify root dynamics using rhizotrons and minirhizotrons over the year.
- Compare various techniques of root image acquisition to choose the best one for each experimental site.
- Examine the influence of soil and air temperatures, soil water potential, soil water volumetric and solar irradiance on root dynamics among different phenological periods.
- Examine the effect of site and different root morphological traits on root survivorship over the year.
- Examine the effect of soil depth on root elongation rate.
- Relate shoot phenology (leaf and stem) to root phenology (structural roots, shallow and deep fine roots).

9. General approach and study sites

In this thesis, we studied belowground processes in relation to above ground phenology of walnut trees (*Juglans* L.) growing in agroforestry systems in France. To check our hypothesis and answer our questions, we conducted our experiments in the field, which gives us a better understanding about how climatic variability impacts root dynamics when roots are in their natural environment. To quantify root growth and mortality (Chapter III) and root growth by soil depth (Chapter IV), we used rhizotrons and minrhizotrons. Rhizotrons and minirhizotrons are a cost-effective, nondestructive way to monitor root growth *in situ*. The advantage of rhizotrons over minirhizotrons, is that, they offer bigger measurable area and better contact between soil and roots, hence, estimates of increases in root length can be better monitored.

10. Chapter arrangement

Chapter II refers to a paper published by the journal Plant Methods entitled (**An evaluation of inexpensive methods for root image acquisition when using rhizotrons**). In this paper we compared the quality of root images obtained using several different types of scanner to measure root growth and root diameter. We also assessed these scanning techniques in conjunction with a fully manual method (tracing onto a plastic sheet) and a fully automated method (time lapse camera). Root length and diameter obtained using each method were correlated with the previously scanned and measured root systems, to see which method gave the best fit. We discussed the results taking into account image accuracy, time spent and cost criteria. Moreover, we demonstrate the advantages or disadvantages of each technique.

Chapter III refers to a paper submitted in Plant and Soil entitled (**Above and below-ground phenological relationships in hybrid walnut growing in agroforests along a climatic gradient**). This paper focused on the temporal evolution of root dynamics in relation with shoot phenology over 21 months along a climatic gradient of temperature and precipitation in France. We examined the influence of different climatic variables and different root morphological traits on root elongation, initiation and mortality of walnut trees (*Jugalns nigra x juglans regia* L.) growing in agroforest systems over the year. We examined also the influence of different classes of diameters and different topological orders on root survivorship. Therefore, we ask the questions: what drives fine root dynamics along a climatic gradient? Are shoot and root are in sync? Results were discussed taking into account the phenological periods over the year.

Chapter IV refers to a paper in preparation titled (**Shoot and deep roots relationships in hybrid walnut growing in Mediterranean alley cropping system**). This paper focused on the temporal evolution of root elongation rate in relation with shoot phenology (leaf and stem) over 21 months in alley cropping agroforestry system in a Mediterranean climate in the south of France. We examined the influence of different climatic variables (soil and air temperatures, soil water volumetric and solar irradiance) on root elongation (in four soil depths until <5 m), stem growth and perennial of walnut trees (*Jugalns nigra x juglans regia* L.) over the year. We related also each organ growth to leaf phenology. Therefore, we asked the questions: what drives deep fine root dynamics growth? Are shoot and root in sync? Are shoot and root under the same controls? Do deep and shallow fine roots have the same drivers? Results were discussed taking into account the aerial phenological periods over the year.

Chapter V The final chapter of this thesis discusses the results and conclusions of the previous chapters, with an emphasis on future studies and perspectives for improving experimental studies in the future.

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Chapter II: An evaluation of inexpensive methods for root image acquisition when using rhizotrons

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Abstract

Background: Belowground processes play an essential role in ecosystem nutrient cycling and the global carbon budget cycle. Quantifying fine root growth is crucial to the understanding of ecosystem structure and function and in predicting how ecosystems respond to climate variability. A better understanding of root system growth is necessary, but choosing the best method of observation is complex, especially in the natural soil environment. Here, we compare five methods of root image acquisition using inexpensive technology that is currently available on the market: flatbed scanner, handheld scanner, manual tracing, a smartphone application scanner and a time-lapse camera. Using the five methods, root elongation rate (RER) was measured for three months, on roots of hybrid walnut (*Juglans nigra*×*Juglans regia* L.) in rhizotrons installed in agroforests.

Results: When all methods were compared together, there were no significant differences in relative cumulative root length. However, the time-lapse camera and the manual tracing method significantly overestimated the relative mean diameter of roots compared to the three scanning methods. The smartphone scanning application was found to perform best overall when considering image quality and ease of use in the field. The automatic time-lapse camera was useful for measuring RER over several months without any human intervention.

Conclusion: Our results show that inexpensive scanning and automated methods provide correct measurements of root elongation and length (but not diameter when using the time-lapse camera). These methods are capable of detecting fine roots to a diameter of 0.1 mm and can therefore be selected by the user depending on the data required.

Keywords: Fine root elongation rate, Flatbed scanner, Handheld scanner, Smartphone, Time-lapse camera

1. Background

Fine root growth, defined as elongation and elongation rate (Barley & Greacen 1967, Germon et al 2016, Jourdan et al 2008) plays an essential role in the cycling and allocation of carbon and nutrients in ecosystems (Gill & Jackson 2000). Due to the inaccessibility of root systems, special techniques are required to investigate the distribution and dynamics of roots, as well as to estimate belowground carbon budgets (Maeght et al 2015, Vogt et al 1998). Today, a number of methods have been used to estimate root growth. These methods can be grouped into indirect and direct techniques (Hendricks et al 2006, Vogt et al 1998), both of which have advantages and drawbacks. Indirect methods include the use of empirical models (Kurz et al 1996), estimations of nitrogen budget and carbon budget (Hendricks et al 2006). Direct methods can be classified into i) destructive techniques such as soil coring (Box & Ramsuer 1993), sequential soil coring (Vogt et al 1998), in-growth cores (Makkonen & Helmisaari 1999, Vogt et al 1998), monoliths (Arnone et al 2000, Gautam & Mandal 2013, Majdi et al 1992) and soil pits (Heeraman & Juma 1993) (Addo-Danso et al 2016, Yuan & Chen 2012), and ii) nondestructive *in situ* methods including isotope quantification (Strand et al 2008), ‘root windows’ or rhizotrons (Bates 1937, Burke & Raynal 1994, Mao et al 2013, Reich et al 1980) and minirhizotrons (Germon et al 2016, Steinaker et al 2010, West et al 2004). Although there are several criticisms concerning these techniques (Strand et al 2008), rhizotrons and minirhizotrons are considered as efficient approaches and are commonly used to characterize fine root growth (Eissenstat & Caldwell 1988, Hendrick & Pregitzer 1996, Majdi 1996). Rhizotrons can be used to monitor (from initiation to mortality) specific root segments at frequent time intervals without significantly impacting root processes (Yuan & Chen 2012). However, the drawbacks of these techniques are related to the cost of installation and potential changes in soil hydrology and physics, which would affect estimates of root production (Hendricks et al 2006, Taylor et al 1991).

Although many studies on root growth using minirhizotrons have been performed (Steinaker et al 2010, Tanner et al 2006), only a small part of the root system can be observed. Techniques for observing root growth include recording root images with digital cameras (Fukuzawa et al 2013, Steinaker et al 2010) and rotating scanners (CID, Inc., WA, USA) (Germon et al 2016), but equipment is expensive. Results from contrasting methods on one single species can also be highly variable (Yuan & Chen 2012). The disparity in results obtained from different methods (Addo-Danso et al 2016, Adu et al 2014, Dannoura et al 2008, Dong et al 2003, Kern et al 2004, Yuan & Chen 2012) has also been attributed to differences in the software used for image analysis (Himmelbauer et al 2004, Lobet et al 2011, Pierret et al 2013).

The quality of images obtained from minirhizotrons and rhizotrons is extremely important for an accurate quantification of root growth through image analysis. The advantage of rhizotrons over minirhizotrons, is that a variety of inexpensive techniques exist for quantifying root growth in the field. Root systems can be measured by tracing onto a transparent plastic sheet (Mao et al 2013), scanning with a flatbed scanner (Dannoura et al 2008, Dong et al 2003, Himmelbauer et al 2004), or a handheld scanner (Pan et al 1998). Scanners have often been considered as the most useful tool for obtaining high quality images, but necessitate the use of a power supply in the field and are not yet fully automated. A detailed comparison of the different types of scanners available has also not yet been performed, especially with regard to the scanners now available as digital applications on smartphones and tablets. Such a comparative study would be highly useful, especially when choosing a particular scanner for a given application and considering its cost, robustness, automation and the quality of the images produced.

With regard to recording automatically images in the field, systems that are independent of an electrical power supply are not yet available, although automated flatbed scanners using 12v batteries for several days have been tested successfully in a teak (*Tectona grandis*) plantation in Lao PDR. (JL Maeght, unpublished data). A fully automated method for measuring root elongation in the field would permit studies of growth in poorly accessible areas or with a poor power supply, as well as detailed measurements of e.g. effects of the circadian clock on root growth *in situ* (Halter et al 1996). To date, most circadian clock studies have been performed in the laboratory on young plants (Halter et al 1996, Willaume & Pagès 2006). Therefore, the necessity of developing a fully automated technique to measure root growth in the field is of major importance.

We compared the quality of images obtained, and the advantages or disadvantages when using several different types of scanner to measure root growth in the field. We focused on inexpensive technology that is currently available on the market and so is accessible to a wide range of potential users. We assessed these scanning techniques in conjunction with a fully manual method (tracing onto a plastic sheet) and a fully automated method (time-lapse camera). Measurements were performed in hybrid walnut (*Juglans nigra×regia* L.) agroforests in France. Results are discussed with regard to quality, time, and cost criteria.

2. Materials and methods

2. 1 Comparison of methods for acquiring images

We examined five methods for acquiring images of root systems:

2.1.1 Flatbed scanner

There are two common types of flatbed scanner, the CIS (Contact Image Sensor) and the CCD (Charge Coupled Device) scanners. A CIS scanner is more compact and requires less power than a CCD scanners and can usually run off battery power or the power from a USB port. CCD scanners, however, provide higher-resolution scans and are capable of scanning with a good depth of field. Accordingly, we used an Epson Perfection V370, high optical resolution of 4800 dpi and CCD technology that relies on a system of mirrors and lenses to project the scanned image onto the arrays. The lid of the scanner can be removed and the scanner connected to the computer via a USB cable and to a 15 V external battery (Fig.1). The scanner can be placed horizontally or vertically. Four horizontal scans and a resolution of 300 dpi are needed for one 50 x 50 cm rhizotron.

2. 1.2 Handheld scanner

Scanners are lightweight (Fig. 1) and portable. We used a Vista Quest HS-500 (USA) to take images at 300 dpi. Scans were taken by moving the scanner manually downwards on the surface of the rhizotron window. Three A4 (21 cm wide and 45 cm long) images are needed for one 50 x 50 cm surface in order to include the borders of our rhizotrons (see section on Rhizotron installation). The images can be saved on a micro Secure Digital memory card and the scanner requires only two AA alkaline batteries to function.

2.1.3 Manual tracing

If no electronic devices are available in the field, roots can be drawn manually with permanent color pens onto a transparent sheet placed over the rhizotron window. Colors indicate different observation times and the date of the observation is noted on the transparent sheet. Transparent sheets are then scanned in the laboratory using e.g. a scanner at a resolution of 300 dpi. (Sharp MX-3640N PCL6, Canada). The manual tracing technique is not usually adequate for measuring root diameter precisely, because root diameter is not known, therefore it is not possible to select a pen with the appropriate point thickness. Nevertheless, manual tracing can be suitable for giving an estimate of root diameter class (e.g. Mao et al (2013) (Mao et al 2013). In this study, we visually estimated root thickness and tried to use pens with the correct point thickness for tracing roots, so that we could compare results with those from the other methods.

2.1.4 Smartphone scanner application

To our knowledge, smartphone scanners have not yet been used for imaging root system growth. We took images using a scanning application on a smartphone (CamScanner INTSIG Information Co., Ltd, Shanghai, China) (version 3.9.5). The CamScanner application automatically detects object borders and removes background noise using image-processing technologies. This software adjusts image details, brightness and contrast and can return processed data in a JPG or PDF format. We also compared several generations of smartphone (iphone6, iphone4, and CAT® S40) to compare the performance of the smartphone technology. To use the application on a rhizotron in the field, the smartphone must be held at a given distance (68 cm in our case) and a fixed scale (tape measure) must be scanned simultaneously to calibrate the scan (Fig. 2).

2.1.5 Time-lapse camera

Although often used to monitor the aerial phenology of vegetation, to our knowledge, time-lapse cameras have not yet been used for automated measurements of root growth and phenology *in situ*. Time-lapse cameras take photographs at regular intervals, determined by the user beforehand. We tested a Cuddeback Attack (U.S.A.) time-lapse camera with flash that takes photographs in color using LED bulbs (Fig. 1). Each camera was placed on a wooden cleat at a distance of 90 cm from the rhizotron. Photographs can be taken every 30 seconds (in our case, we took one photograph at 2 a.m. and at 12 hour intervals thereafter). Time-lapse cameras can run for several months on an Alkaline battery (C (LR14) 1.5V) without any human intervention.

2.2 Comparison of methods

2.2.1 Test 1: previously scanned and measured root systems

To allow for a fully comprehensive comparison of data between scanning, manual and automated methods, we tested each method on previously scanned and measured root system (n = 35), and likewise on a measuring tape placed in different positions (Fig. 3) of known dimensions in a rhizotron (50 x 50 cm). The scanned root systems were measured using four methods: flatbed scanner, handheld scanner, smartphone scanners and the time-lapse camera. Data were imported to the SmartRoot software.

2.2.2 Test 2. Measurements in rhizotrons using scanners and manual tracing

We performed measurements at Le Beil, Madic, in the Cantal region, France (45°22'7.95"N, 2°28'1.46"E) (see section on study site for more details). We started the observations in October 2014 and fine root growth was measured every month from April to June 2015 using four methods: flatbed scanner, handheld scanner, smartphone scanner (iphone4) and the manual tracing (n= 25). Walnut fine roots are quite thick and so roots ≤ 4 mm in diameter were classed as fine roots.

2.2.3 Test3. Measurements in rhizotrons using a time-lapse camera

A third set of measurements was performed at Cormont, in the Pas de Calais region, France (50°33'27.87"N, 1°44'3.08"E), (see section on study site for more details). Root growth was monitored in six rhizotrons twice a day from May to September. We focused our study on 21 roots growing over a period of 10 days for un easier understanding and comparison of results.

2.3 Image analysis

Once images of root growth had been acquired, we conducted analyses of images using the semi-automated SmartRoot software (Lobet et al 2011). SmartRoot is an operating system independent freeware based on ImageJ and uses cross-platform standards (RSML, SQL, and Java) for communication with data analysis softwares (Lobet et al 2011, Mathieu et al 2015). Before analyzing roots with SmartRoot, when necessary, images need “stitching” together (e.g. with Adobe Photoshop CS3 software), if several have been taken (when the rhizotron surface area was greater than the field of the scanner). In our case, we transformed all images to 8 bit gray scale and then inverted them using ImageJ software so that roots were darker than the background of the image. The length and diameter of each root produced during one interval time (i.e. one month) were calculated for each rhizotron. Before analyzing a new sequence of images, SmartRoot provides the user with an icon to import the traces of the same roots from the previous image data file to superimpose them on this new image, which helps root elongation. This preceding image also helps determine whether the root is live (usually cream in color) or in a phase of senescence (shriveled, transparent or turning black) (Anderson et al 2003, Germon et al 2016, Graefe et al 2008, Huck & Taylor 1982, Tierney & Fahey 2002). We declared a root dead when it became completely black in color.

2.4 Study site

We measured fine root growth *in situ* in two agroforests. One was located at Le Beil, Madic, in the Cantal region, France (45°22'7.95"N, 2°28'1.46"E) at an elevation of 530 m, hereafter termed 'continental' climate. The agroforest comprised three transplanted tree species: hybrid walnut (*Juglans major (MJ209)* × *Juglans regia* L.), cherry (*Prunus avium* L.), sycamore maple (*Acer pseudoplatanus* L.) at 12 m x 8 m tree spacing and intercropped with permanent pasture (ovine or bovine pasture). All national guidelines and legislation were complied with when using these cultivars. Mean diameter at breast height (DBH) of all walnut trees at the site was 0.20 ± 0.02 m and mean height was 12.09 ± 1.30 m. Data are means ± standard error. All trees were planted in 1994 at a density of 100 trees ha^{-1}. Hybrid walnut at this study site starts leafing in early May and shedding in mid-November. The climate is continental with a mean annual temperature of 9.95°C and a mean annual rainfall of 1174 mm (Météo France). The soil is silty and not deep, attaining an average maximum depth to bedrock of approximately 110 cm, on a 5° - 10° slope.

The second agroforest was located at Cormont, in the Pas de Calais region, France (50°33'27.87"N, 1°44'3.08"E), hereafter termed 'oceanic' climate. The site is at an altitude of

40m. The climate is oceanic, with a mean annual temperature of 11°C and a mean annual rainfall of 777.9 mm (Météo France). Tree species comprised hybrid walnut (*Juglans nigra* × *regia* L.) and Maple (*Acer laurinum* L.) at 13 x 7.5 m tree spacing intercropped permanent pasture (ovine pasture). All trees were planted in 1999. The soil is silty clay and < 2.5 m deep. The site is next to La Dordonne River. Mean DBH of walnut trees at the site was 0.30 ± 0.03 m and mean height was 14.75 ± 3.50 m. Hybrid walnut at this site starts leafing in early May and shedding in mid-November.

2.5 Rhizotron installation

In the continental agroforest (Madic), we dug eight (1 m x 1 m x 1 m) trenches by hand in three rows of trees. Each trench was at a distance of 2 m from the nearest tree stem. Eight rhizotrons, or root windows (50 cm long x 50 cm wide x 0.5 cm thick), were installed. In the oceanic agroforest (Cormont), soil was deep (4 m) and comprised four (2 m long x 1 m wide x 2 m depth) trenches in one row of trees placed at 2 m from the nearest tree stem. One rhizotron was installed on two opposing faces of the trench (n = 8 rhizotrons in total). All rhizotrons were placed vertically at an angle of 15° from the face of the profile. This angle will permit the roots to grow downwards due to positive geotropism (Huck & Taylor 1982, Mao et al 2013). Where the rhizotrons were to be placed on the trench, we gently removed the soil to make a flat surface and cut all roots on the profile with secateurs. The soil removed during the digging of the trenches was kept aside, and then sieved through a 5 mm size sieve and air-dried for several hours. The sieved and air-dried soil was then poured into the space between the window and the soil profile and slowly compacted using a wooden plank. Each rhizotron was covered with foil backed felt insulation and black plastic sheeting to protect roots from light and temperature variations. All trenches were then covered with wooden boards and corrugated plastic to avoid damage from passing animals and to prevent direct rainfall and sunlight onto the rhizotrons. In the first three months after installation, no root growth was recorded to avoid over estimations of root growth (Strand et al 2008).

2.6 Root indicator calculation

We used the following method to estimate root elongation rate:

individual root growth was evaluated by calculating the difference between the root length at t_{-1} and at t. To determine the daily root elongation rate (RER), the mean of all individual root lengths produced between time t and t_{-1} was divided by the duration of the corresponding period (Germon et al 2016). According to the literature, the characterization of dead roots is not obvious, particularly behind a transparent window (Tierney & Fahey 2002). We considered root as live when it had a cream color and dead when it had turned black with no growth between two or more successive sessions until the last observation date occurred (Germon et al 2016).

The equation we used to calculate RER was:

$$RER_{t\text{-}1,\,t} = \frac{len._{t} - len._{t\text{-}1}}{Pt\text{-}t_{-1}}$$

Where, $RER_{t\text{-}1,\,t}$ is the daily root elongation rate; $ln._{t\text{-}1}$ and $ln._{t}$ are the lengths of the root n at inventory time t_{-1} and t, respectively; $p_{t\text{-}1,\,t}$ is the period between inventory time t_{-1} and t (d).

At the oceanic site, as we took two photos per day (using the time-lapse camera), we aimed at testing whether our method could be used to estimate differences in RER during the day and at night (Halter et al 1996). Each root was evaluated by calculating the difference between the root length at $t._{n1}$ and at $t._{d1}$ for root growth during the daytime and between $t._{d2}$ and at $t._{n1}$ for root growth during the night.

$$RE_{day} = len._{tn1} - len._{td1}$$

$RE_{night} = len._{td2} - len._{tn1}$

Where RE is root elongation during12 hours (in mm); $len._{td1}$, $len._{tn1}$ and $len._{td2}$ is the length of the root n at inventory daytime$_1$, night time$_1$ and daytime$_2$ respectively.

2.7 Semi-quantitative scoring decision matrix

Three parameters were taken into account to evaluate the five methods: (i) accuracy (image quality and resolution, deformation and contrast), (ii) effectiveness (time, expenditure and labour) and (iii) adaptability (ease of use in field and necessity of accessories).

2.8 Statistical analysis

Root length and diameter obtained using each method were correlated with the previously scanned and measured root systems, to determine which method gave the best fit. Similarly, results from different generations of smartphones were compared. We then calculated relative values for cumulative length, mean diameter and RER, with regard to the flatbed scanner (reference value), which we assumed gave the most accurate dimensions (Dannoura et al 2008, Dong et al 2003). To calculate the relative value, we divided the value obtained for individual roots (using each method) by that obtained using the flatbed scanner.

A Shapiro-Wilk test was performed before each test to ensure if the investigated indicator followed a normal or non-normal distribution. Homogeneity of variances was checked. For data not normally distributed, analyses were followed by a Kruskal-Wallis Test for each factor. A post-hoc analysis between root diameters was performed using the Nemenyi test of Kruskal Wallis at $p<0.05$ to determine which levels of the independent variable differ from every other level. All analyses were performed using R software, Version 2.15.3 (R Development Core Team 2013) at a significance level of <0.05.

3. Results

Test 1. Previously scanned and measured root systems

When images from the different generations of smartphone were compared, no significant differences were found with regard to root length and diameter between any models. When all methods (except for manual tracing) were compared together, there were no significant differences in the relative cumulative length of previously scanned and measured root systems. However, the time-lapse camera significantly overestimated the relative mean diameter of previously scanned and measured root systems compared to the other three methods ($p < 0.001$, Fig. 4). Although our time-lapse cameras had a high resolution (20 megapixels), this overestimation was likely due to the low optical resolution leading to a poorer quality of image. The SmartRoot software estimates the diameter of the root by diagonally measuring nodes along each root, but if the image is of low quality, SmartRoot will not be able to detect and distinguish correctly the border of the root (Fig. 3).

Test 2. Measurements in rhizotrons using scanners and manual tracing

There were no significant differences in relative RER between the four scanning methods (Fig. 5). However, manual tracing on transparent sheets significantly overestimated the relative mean diameter of roots ($p < 0.001$, Fig. 6). This overestimation was due partly to human error, as the Plexiglas window and the plastic sheet resulting in multiple layers, obscuring the root outlines, as well as the pens being either too thick or too fine for matching root diameter exactly.

Test 3. Measurements in rhizotrons using a time-lapse camera

Roots were found to elongate up to 20 mm in a single day (Fig. 5) and when cumulated over a period of 10 days, up to 48 mm of growth occurred. When comparing root elongation between day and night (with a period of 12 hours between the two measurements) no overall significant differences were found over the 10 day period examined (Fig. 7) or over the whole lifetime of individual roots (Fig. 8). This method allowed therefore also us to estimate differences in root elongation between day and night.

4. Discussion

Studies on root growth have been numerous over the last decade and a significant progress in evaluating root morphology has been observed. However, research remains challenging and costly especially in the natural environment. Many nondestructive methods, such as rhizotrons and minirhizotrons (Germon et al 2016, Steinaker et al 2010, West et al 2004) have been developed to overcome some of the limitations of observing root systems in the natural environment and to offer direct and repeated observations of root system morphology. Image quality obtained from rhizotrons and minirhizotrons is crucial for an accurate quantification of root growth through image analysis.

We show that all five methods for imaging root systems can be used to determine root length, but that if accurate measurements of root diameter are required, a scanner must be used, and not the time-lapse camera nor manual tracing. The smartphone scanning application was found to be perform best overall when considering image quality. Images were sharply contrasted, of high resolution and deformation was minimal. The application was free for smartphones and did not need any accessories. Only a short amount of time was spent in the field acquiring data and image treatment can be carried out directly in the application (Table 1). Another advantage of the smartphone is its genericity and wide community of developers and any of the models tested could be used, with no consequences on results obtained. Many third party hardwares (such as additional lenses, holders, batteries) and software tools (automated cloud backup, automated geographical tagging, etc.) are available, often at minimal cost. It should also be noted that the quality of the smartphone camera and the lifetime of batteries have been constantly improved by manufacturers, probably at a much faster rate than for specialized equipment. In contrast, the flatbed scanner has many accessories so it is not easily transportable and needs four images for one 50 x 50 cm rhizotron, therefore much time is needed in the laboratory to merge images before analyzing them. Additionally, automatic flatbed scanners have not been developed yet. Thus, the scanner cannot automatically acquire images in the field over a long period because of the need for a power supply in the field. However, the method was rapid, easy to use and inexpensive. Image quality was very high as also found by other users (Dannoura et al 2008, Dauer et al 2009, Dong et al 2003) (Table 1). Likewise, the advantage of the handheld scanner is that it is quick, portable and the images are of good quality (Pan et al 1998). Three images were needed for one 50 x 50 cm surface with this method, and the major constraint with this scanner is the size of images (29.7 x 21.0 cm), so more time is needed for merging images manually before analyzing them (Table 1). The distinct advantage of tracing onto transparent sheets is their inexpensive price (Table 1), but inaccuracies due to human error and optical effects occur, resulting in an overestimation of root diameter. As scanning methods are not yet automated for use in the field, the main advantage of the time-lapse camera is that it can be left in place for several months without any manual intervention and it is relatively inexpensive (Table 1). However, the quality of images taken with the time-lapse cameras was poor and a certain amount of reflection occurs due to the flash, leading to an ultimate overestimation of root diameter. The low quality of images taken is because the lenses have less optical resolution compared to e.g. a smartphone camera, resulting in blurred photos. The optical resolution represents the physical resolution to resolve detail in the object that is being imaged via an imaging system. Smartphone cameras and digital cameras have been developed to be capable of defining the smallest discernible detail in an image, resulting a better spatial resolution which states the clarity of an image and this resolution refers to the number of pixels used to construct the image. This spatial resolution depends on properties of the system creating the image, not just the pixel resolution (pixels per inch). For example, in our study, although the smartphone had less resolution (5 megapixels) than the time-lapse camera (20 megapixels), it produced a better quality of image. This quality is because the smartphone imaging system can detect spatial differences and this spatial resolution can be influenced by diffraction, aberrations, imperfect focus, lens, size of the sensor and other imaging system components. Furthermore, as the automated time-lapse camera was programmed to autofocus mode, the camera may focus on the wrong part of the image depending on the environment where the image is captured. We successfully used the time-lapse camera to compare root elongation during the day and at night, but no significant differences were found. Nevertheless, from Figs 7 and 8, it can be seen that some roots grow mostly at night and others grow mostly during the day, although the reason for this disparity between roots is not known.

To the best of our knowledge, the smartphone scanning application and an automated time-lapse camera have never been used to measure root growth in the field. Both methods are inexpensive and easy to use, especially compared to more sophisticated techniques such as minirhizotron scanners. The advantage of rhizotrons over minirhizotrons is that the above variety of inexpensive techniques exist worldwide for quantifying root growth in the field. The equipment needed to observe and record color video pictures of roots in minirhizotrons (Box & Ramsuer 1993, Patena & Ingram 2000) is commercially available at a cost of approximately 10 000 euros for one circular scanner (Germon et al 2016) or one camera video. Additionally, the field of vision in a minirhizotron is small (20 x 20 cm) and is not suitable for heterogeneous forest stands, where the spatial position of roots of different diameter classes can lead to root-free patches in the soil. The 50 x 50 cm rhizotrons we used in our study enables more tree roots to be captured in one image, thus increasing statistic robustness.

Table. 1 Multiple criteria evaluation/ Semi-quantitative scoring decision matrix.

Method	Smartphone application	Flatbed scanner	Handheld scanner	Camera	Transparent sheet
Image quality	***	***	***	*	-
	High	High	High	Low	
Deformation	***	***	***	**	**
- length	No	No	No	No	No
- diameter	No	No	No	Yes	Yes
Time	****	**	***	****	*
/ rhizotron	40 sec	12 min	1min ± 1min	0 s	12 min
/8 rhizotron	7 min	96 min	16 min		95 min
Cost	**	**	**	*	****
	Phone100-700€ Apps free	150 €	80 €	190 €/rhizotron	
Usage in field	****	**	***	***	*
	Easy/1 person	Difficult/2 persons	Easy/1 person	Autonomous	Difficult/1 person
Accessories	***	*	***	***	***
	No	Yes/battery and PC	No	No	No
Time between visits	**	**	**	***	**
	2 to 4 weeks	2 to 4 weeks	2 to 4 weeks	4 to 5 months	2 to 4 weeks
Time to treat images before analysis	***	*	*	*	***
	0 s	3.30 min/image	4 min/image	3 min	0 s

5. Conclusion

We tested several methods for monitoring root growth and acquiring images in rhizotrons in the field. Our results show that scanners and time-lapse cameras provide correct measurements of root growth and length in the field but users should be aware of possible artifacts. Time-lapse cameras overestimate root diameter but are useful for taking frequent images of root elongation in the field over several months, without any manual intervention. Taking into account image accuracy, time spent and cost, we found the smartphone scanner to be the optimal method for monitoring root growth in the field. Future generations of smartphones could scan images and transfer data automatically, with a minimum of human intervention, thus improving the methodology. Likewise, the development of digital time-lapse cameras with a higher optical resolution, or similar to the optical resolution of smartphones, should also be undertaken.

6. Abbreviations

RER: Root elongation rate, RE: Root elongation.

7. General informations

Funding

Funding for a PhD bursary was provided by Campus France and the Kurdish Institute, France (AM) and fieldwork was funded by the INRA metaprogram AAFCC (Adaptation of Agriculture and Forests to Climate Change), France.

Authors' contributions

AM, YM and AS designed the study. AM, YM, AS, ZM and MR conducted fieldwork. AM, ZM and GL contribute to data analysis. AM and AS wrote the paper with contributions from YM, ZM, JLM and GL. All authors read and approved the final manuscript.

Acknowledgements

Thanks are due to François Pailler and Stéphane Fourtier (INRA) for technical assistance, to Camille Béral for help finding field sites, to Jérôme Nespoulous (INRA) for his help with field and laboratory work and to the farmers M. Queuille and M. Becue for letting us work in their agroforests.

Figure 1

Four different methods were used to take images of walnut tree roots in rhizotrons: a) flatbed scanner, b) handheld scanner, c) smartphone scanning application and d) time-lapse camera. In d), the time-lapse camera and rhizotron were placed into insulated boxes so that variations in temperature did not affect root growth. In the other rhizotrons, insulating material was placed over the rhizotron Plexiglas pane and removed before scanning.

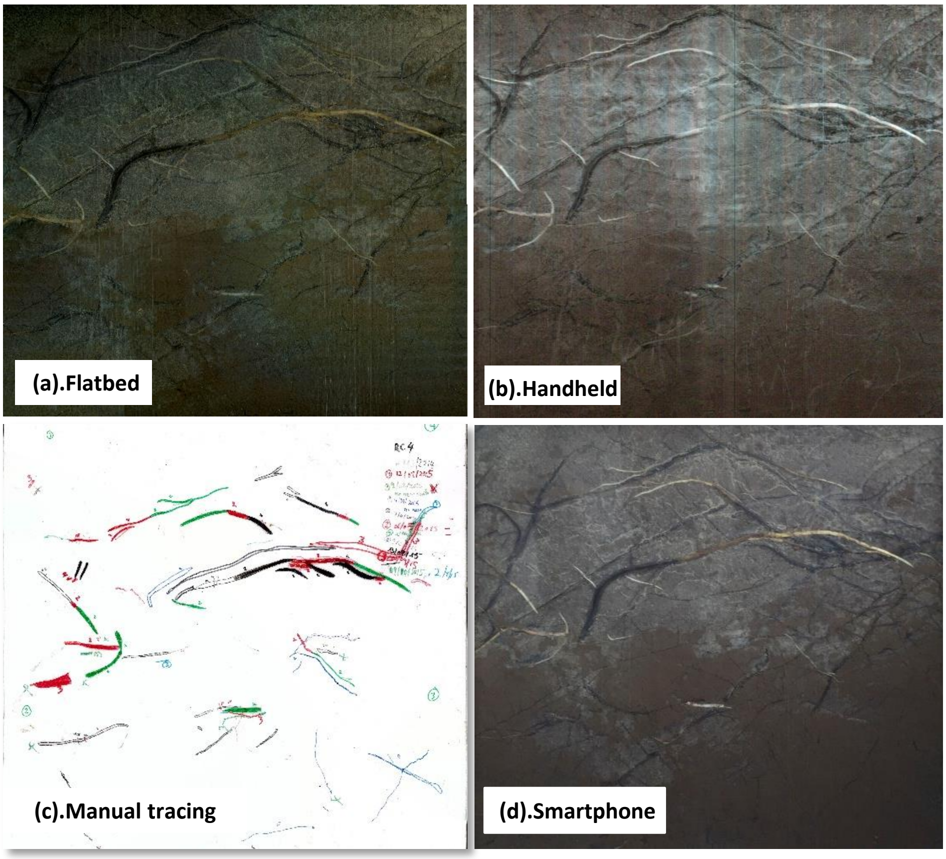

Figure 2

Examples of images taken by a) flatbed scanner, b) handheld scanner, c) manual tracing on a transparent sheet and d) smartphone scanner in the same rhizotron at the same date.

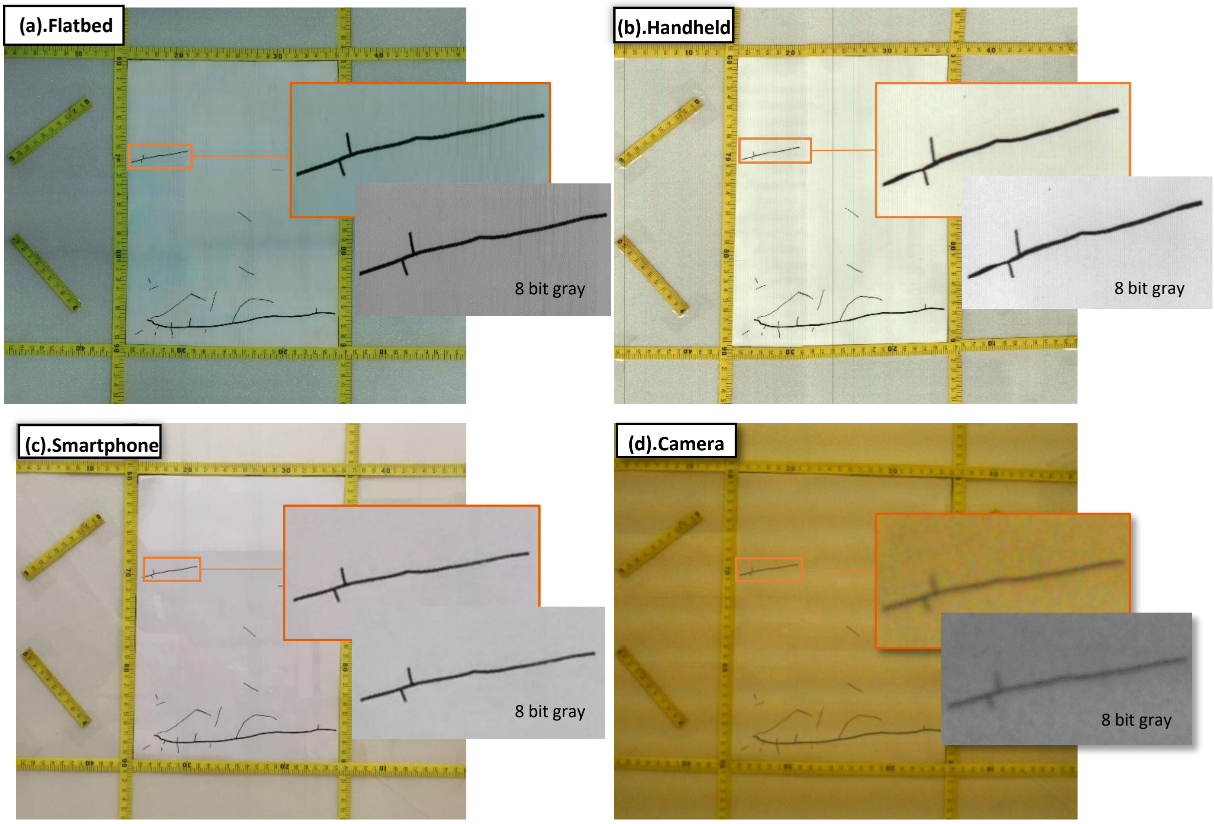

Figure 3

We tested the accuracy of a) flatbed scanner, b) handheld scanner, c) smartphone scanner and d) time-lapse camera by measuring root systems of known dimensions in the laboratory. A zoom of one root shows the quality of the images taken using each method before and after transforming the image to an 8 bit gray scale.

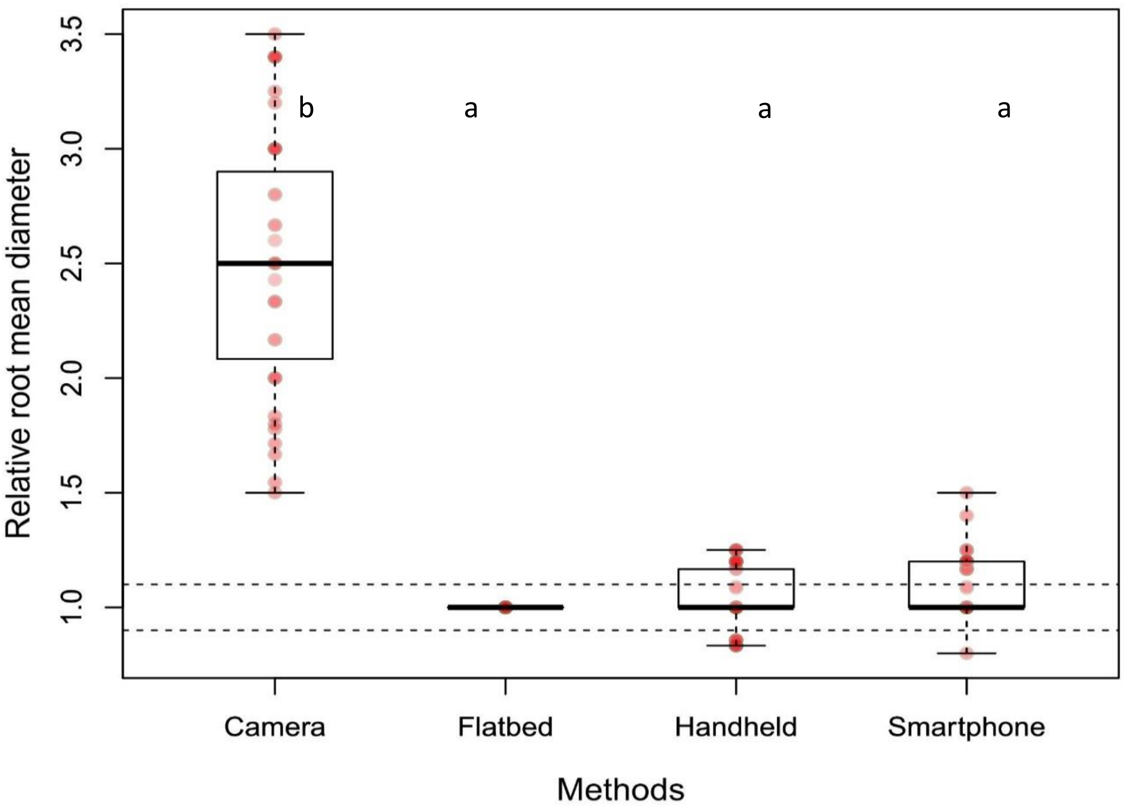

Figure 4

Comparison of the relative mean diameter (mm) of roots from root systems of known dimensions. The time-lapse camera significantly overestimated the diameter of roots compared to the three scanning methods ($p < 0.001$). Each circle represents diameter data for one root. Differences in shading intensity of circles indicate that one or more data points are superimposed. The lower edge of the box corresponds to the 25th percentile (Q1) data point, while the top edge of the box corresponds to the 75th percentile data point (Q3). The line within the box represents the median and the whiskers indicate the range of the data. The two horizontal dashed lines represent an interval of 10% above and below the median of the reference method (flatbed scanner). Different letters above the boxplots indicate statistically significant differences ($p < 0.05$) between methods.

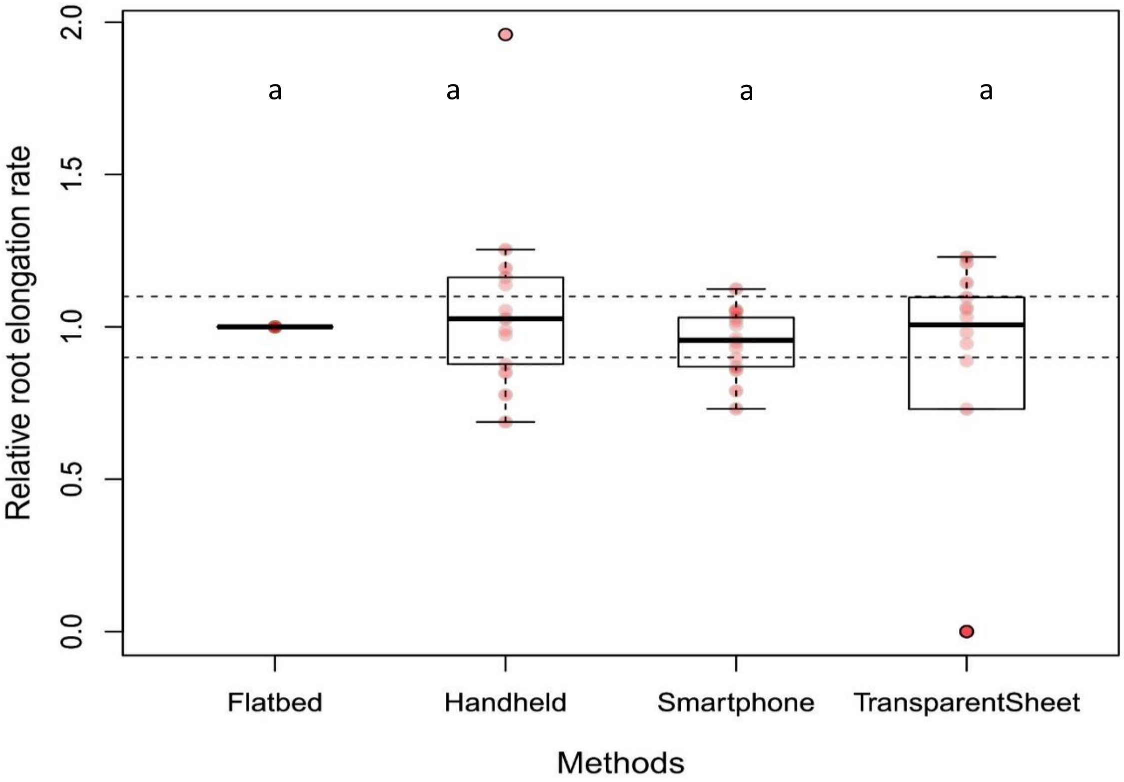

Figure 5

Comparison of the relative root elongation rate (RER) between the different image acquisition methods. Each circle represents RER for one root. Differences in shading intensity of circles indicate that one or more data points are superimposed. There were no significant differences in RER between the four methods. Each circle represents diameter data for one root. Differences in shading intensity of circles indicate that one or more data points are superimposed. The lower edge of the box corresponds to the 25th percentile (Q1) data point, while the top edge of the box corresponds to the 75th percentile data point (Q3). The line within the box represents the median and the whiskers indicate the range of the data. The two horizontal dashed lines represent an interval of 10% above and below the median of the reference method (flatbed scanner). Different letters above the boxplots indicate statistically significant differences ($p < 0.05$) between methods.

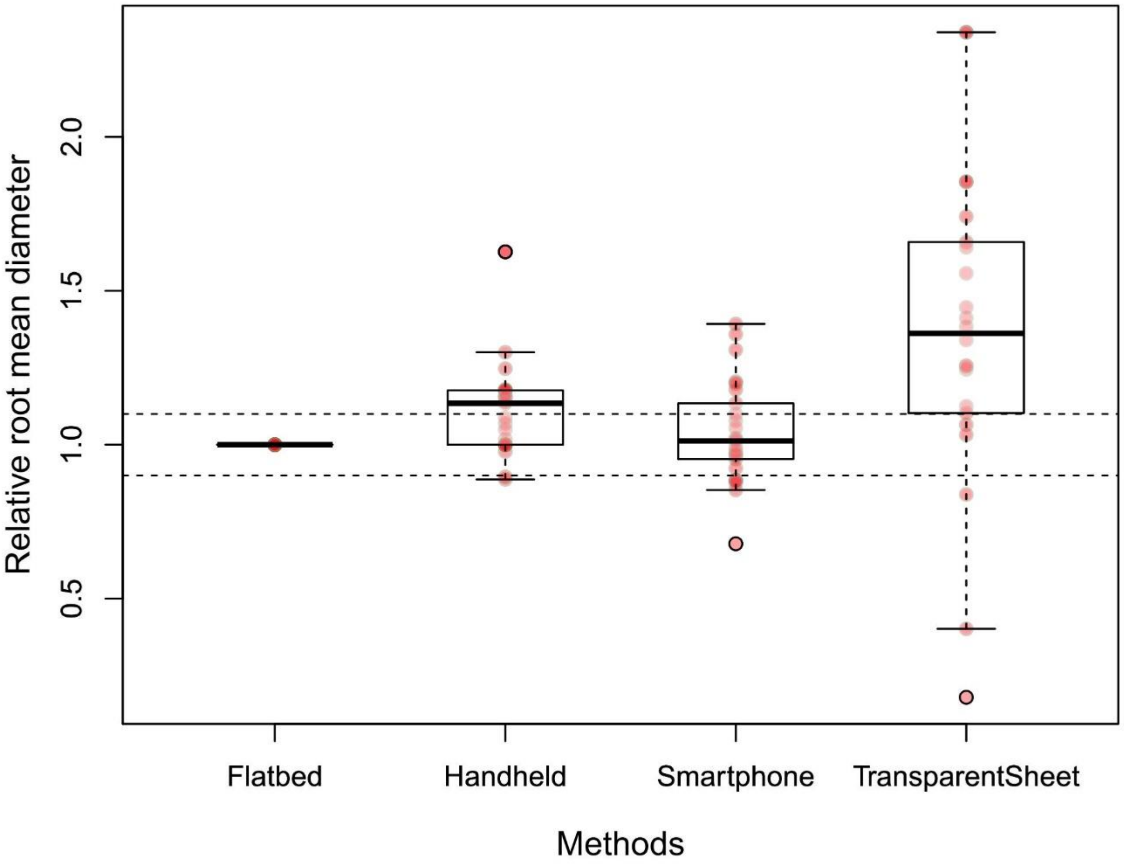

Figure 6

Comparison of the relative mean diameter (mm) of roots growing in rhizotrons *in situ*. Manual drawing on transparent sheets significantly overestimated the mean diameter of roots compared to the three scanning methods ($p < 0.001$). Each circle represents diameter data for one root. Differences in shading intensity of circles indicate that one or more data points are superimposed. The lower edge of the box corresponds to the 25th percentile (Q1) data point, while the top edge of the box corresponds to the 75th percentile data point (Q3). The line within the box represents the median and the whiskers indicate the range of the data. The two horizontal dashed lines represent an interval of 10% above and below the median of the reference method (flatbed scanner). Different letters above the boxplots indicate statistically significant differences ($p < 0.05$) between methods.

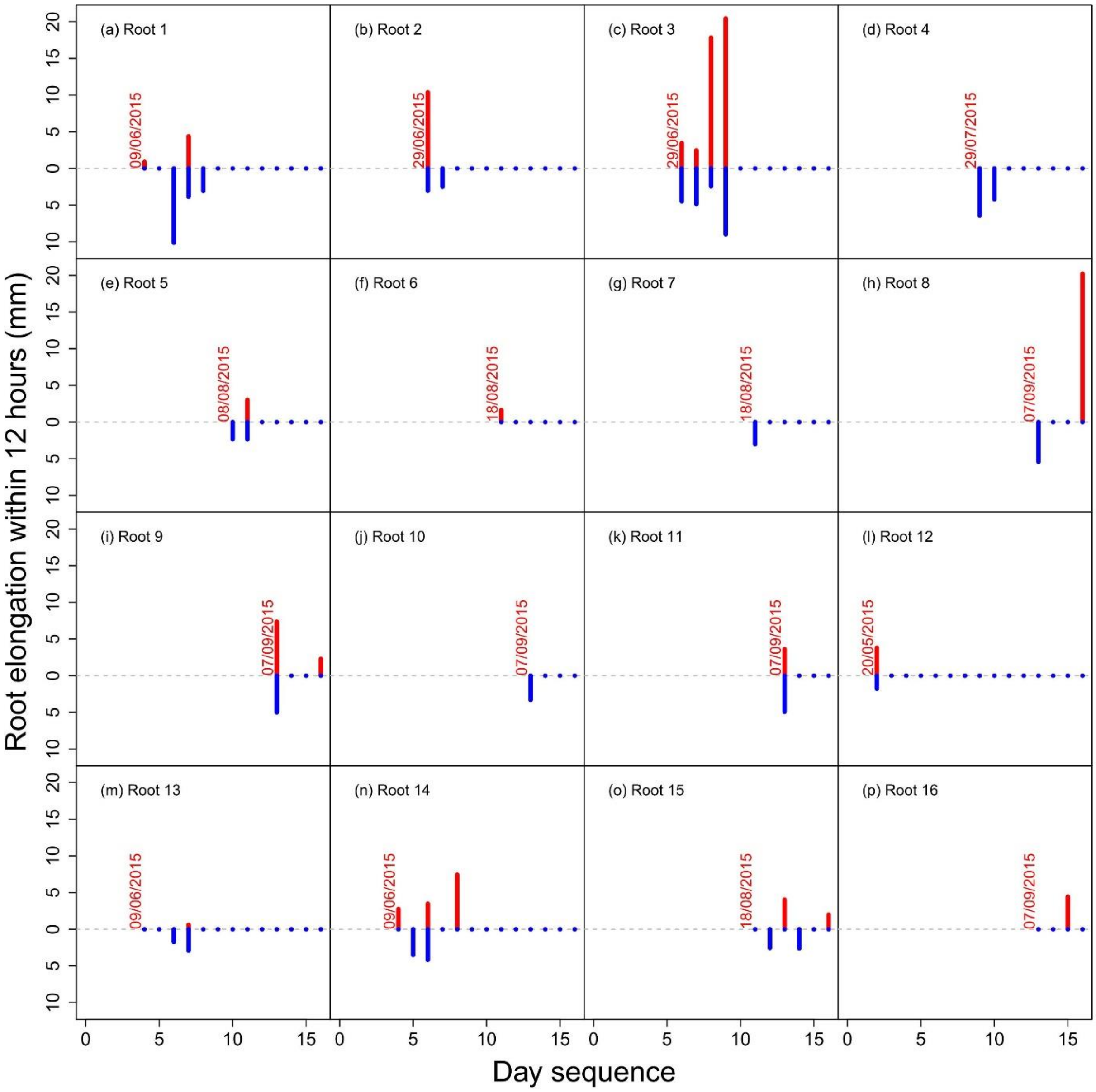

Figure 7

Root elongation reached 20 mm day^{-1} in certain roots. No significant differences were found in elongation between day and night over an interval of ten days measured using a time-lapse camera. The red color represents root elongation during the day and the blue color represents root elongation during the night. If no bars are present, roots did not grow during that period (even though they were still alive). The date above the first data point indicates when root growth started.

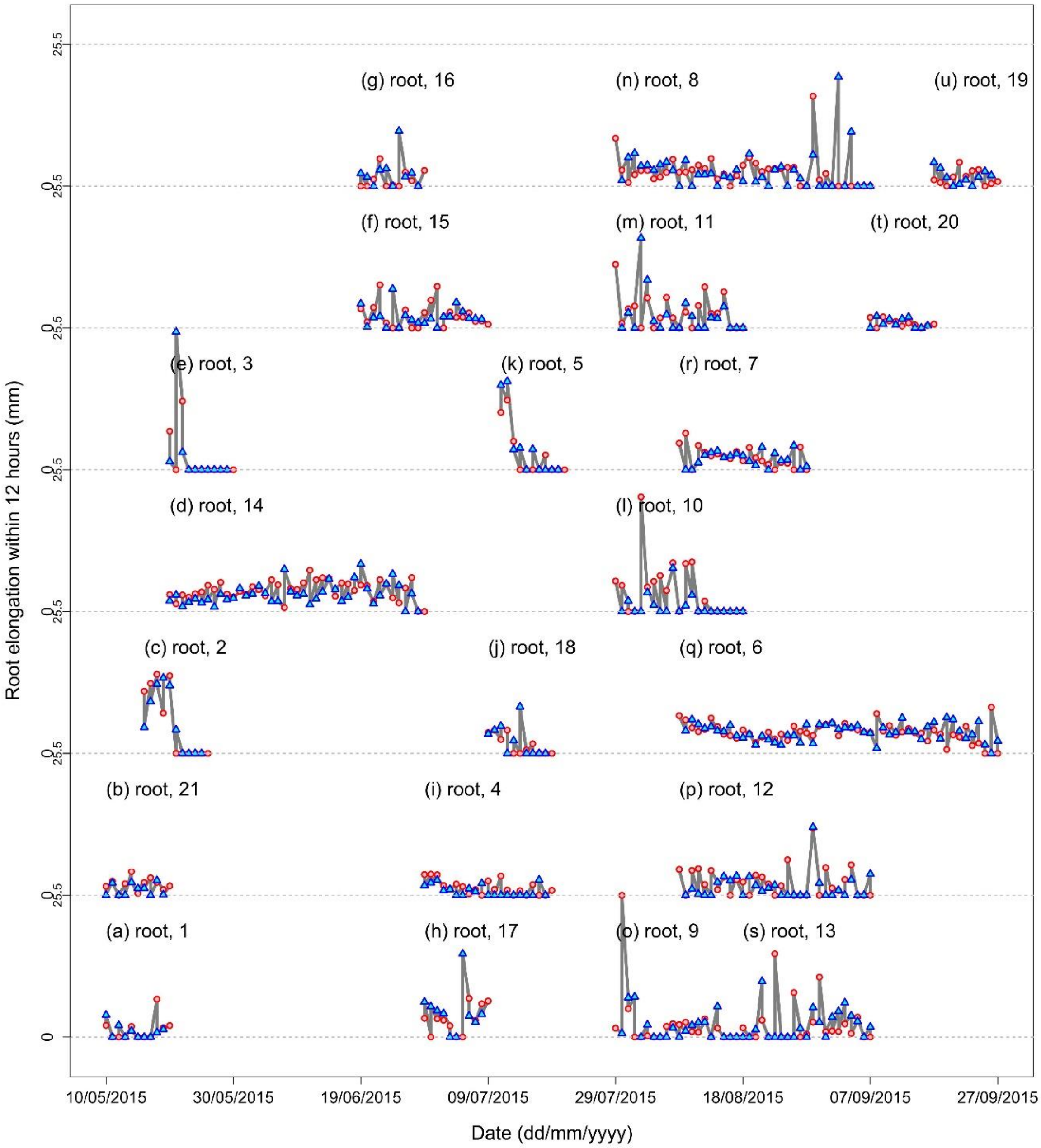

Figure 8

Root elongation (mm) in the daytime and at night, estimated using a time-lapse camera over a period of 10 days. Symbols: circles = root elongation during the day, triangles = root elongation during the night. For (a-s), each graph represents the elongation rate of one root randomly selected from a rhizotron throughout its entire lifespan.

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Chapter III: Above and belowground phenological relationships in hybrid walnut growing in agroforests along a climatic gradient

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Abstract

Plant phenology is a sensitive indicator of plant response to climate change. Belowground processes play an essential role in ecosystem carbon and nutrient cycling. Observations of events belowground for most ecosystems are difficult to obtain and hence less characterized than those aboveground. Very little is known about the relationship between shoot and root phenology, especially in the field. We examined the influence of environmental factors and root morphological traits on fine root production and mortality in relation with shoot phenology in walnut trees (*Juglans* L.) growing in three temperate agroforestry systems along a latitudinal gradient (oceanic, continental and Mediterranean climates). Rhizotrons were installed for 21 months to monitor root dynamics. Root growth was not synchronous with leaf phenology in any climate or either year, but was synchronous with stem growth during the late growing season. One distinct flush of root growth was observed during the aerial growing season but growth was negligible during the rest of the year. Maximal mean root elongation rates (RER) ranged from 2-5 mm during the late growing season depending on climate. Mean RER was driven by soil temperature measured in the month preceding root growth in the climate site only. However, mean RER was significantly correlated with mean soil water potential measured in the month preceding growth at both Mediterranean (positive relationship) and oceanic (negative relationship) sites only. Root diameter had no effect on RER. Mean root initiation quantity (mRIQ) was significantly higher at both continental and Mediterranean sites than the oceanic site. Soil temperature was a driver of mRIQ during the late growing season at continental and Mediterranean sites only. Mean RMQ increased significantly with decreasing soil water potential during the late aerial growing season at the continental site only. Mean root longevity at the continental site was significantly greater than for roots at the oceanic and Mediterranean sites. Roots in the 0-1 mm and 1-2 mm diameter classes lived for significantly shorter periods compared to those in the 2-5 mm diameter class. First order roots lived longer than lateral roots at the Mediterranean site only and first order roots in the 0-1 mm diameter class had 44.5% less risk of mortality than that of lateral roots for the same class of diameter. We conclude that factors driving root RER were not the same between climates. However, air temperature was the best predictor of root initiation at all three sites, but drivers of root mortality remained largely undetermined.

Key words

Rhizotron, phenology, root elongation, root initiation, root mortality, root survivorship

1. Introduction

Climate models predict that an increase in atmospheric CO_2 concentration, precipitation and temperature could affect many biological phenomena and increase the frequency and magnitude of extreme weather events (Solomon et al 2007). Changes in plant phenology are considered to be a very sensitive and observable indicator of plant responses to climate change (Steinaker et al 2010) (Morin et al 2010). The timing of above and below ground phenological events is important to assess ecosystem function and plant productivity (Fridley 2012) (Richardson et al 2006). Aboveground phenological events include bud burst, leaf expansion and leaf fall, all of which have attracted attention because of the role they play in determining species' responses to climate change (Diez et al 2012). However, observations of important events belowground, including timing of root initiation, peak growth, survivorship and cessation of growth, for most ecosystems are more difficult to obtain and hence are less characterized especially in natural soil conditions.

Fine root dynamics, defined as elongation rate (Germon et al 2016, Jourdan et al 2008), production and mortality (Hendrick & Pregitzer 1993b) (Mao et al 2013b) (McCormack et al 2014), turnover (Anderson et al 2003), survivorship (Anderson et al 2003) (Kern et al 2004) and senescence (Huck et al 1987), play an essential role in ecosystem nutrient cycling and the global carbon budget (C) cycle (Vogt et al 1995, 1998) (Gill & Jackson 2000) because they regulate the storage of large quantities of carbon. It is widely acknowledged that fine root phenology could be strongly influenced by different factors (biotic and abiotic) throughout the year, and these factors alter patterns of root growth and longevity. Several studies found strong effects of abiotic factors on root elongation, such as soil temperature (Kuhns et al 1985) (Wan et al 2002) (Tanner et al 2006) (Steinaker & Wilson 2008) (Steinaker et al 2010) (Coll et al 2012), soil water content (Green et al 2005) (Metcalfe et al 2008, Misson et al 2006) (Block et al 2006) or air temperatures (M'bou et al 2008) (Tierney & Fahey 2002;

Fukuzawa et al 2013). For example, Mao et al, (2013) studying *Picea abies* and *Abies alba* in montane and subalpine forest ecosystems have found that high rates of root elongation occurred when soil temperature was >1°C. Germon et al, (2016), studying walnut trees in Mediterranean climate also found that root elongation rate (RER) was positively and significantly correlated with mean soil temperature. In contrast, other studies have found no correlations with abiotic factors (Hendrick & Pregitzer 1993a, Hendrick & Pregitzer 1993b, Hendrick & Pregitzer 1996a) (Joslin & Wolfe 1998, Joslin et al 2000) and suggest that endogenous factors, such as growth regulators (McAdam et al 2016), photoassimilate transport and photosynthates availability (Sloan et al 2016) (Tierney & Fahey 2002) (Joslin et al 2000), are the main drivers of growth. Abramoff and Finzi, (2015) reviewed that endogenous cuing and allocation of stored carbohydrates were dominant drivers of root growth in Mediterranean trees. Radville et al, (2016), studying Arctic shrub-graminoid communities, showed that root growth occurred in near freezing temperatures and was not driven by soil temperature. Other studies related root elongation to diameter classes, for example (Mao et al 2013c) showed that daily RER of *Picea abies* and *Abies alba* in montane and subalpine forest ecosystems was lower in fine roots than in thicker roots.

While air temperature may be the most important environmental factor controlling the timing of aboveground growth, as reported by a number of studies (Menzel 2003, Radville et al 2016a, Wielgolaski 1999), the drivers of belowground phenology are less clear. We also have a poor understanding of the relationship between root growth and leaf phenology (Pregitzer et al 2000) (McCormack et al 2014) (Abramoff & Finzi 2015) and how this relationship is affected by abiotic factors. More effort is needed in understanding such relationships. It is commonly assumed that root and shoot growth are asynchronous (Steinaker et al 2010) (Abramoff & Finzi 2015, Sloan et al 2016) with a peak of root growth in the early and late spring (Contador et al 2015) (Germon et al 2016) or in the summer (Psarras et al 2000).

Recent studies have indicated that root growth can occur in a single flush but often occurs in multiple flushes throughout the growing season (Reich et al 1980) (Harris et al 1995) (Steinaker et al 2010) depending on resources availability either during a single seasonal pulse or multiple periods of favorable environmental conditions (McCormack et al 2014). In temperate forests, leaf growth occurs several weeks before root growth (Steinaker & Wilson 2008) (Abramoff & Finzi 2015) (Harris et al 1995). While Radville et al, (2016) in an arctic climate, and Abramoff and Finzi, (2015) reviewing data from a subtropical climate, have shown that root growth precedes shoot growth by several weeks to months (Radville et al 2016b) (Broschat 1998).

Current climate models assume that root and shoot growth are synchronous and controlled by the same factors as also found by (Germon et al 2016). However, several studies have suggested that it is necessary to consider shoots and roots separately because the drivers of each one are not the same (Abramoff & Finzi 2015) (Blume-Werry et al 2016). However, simultaneous measurements of root and leaf phenology remain limited.

Fine root turnover provides considerable amounts of carbon and nitrogen to the soil (Luke McCormack et al 2012) (Cox et al 1977) (Hendrick & Pregitzer 1993b) (Wells & Eissenstat 2001), often exceeding the amounts returned by leaf litter fall in some ecosystems (Vogt et al 1986). Fine root lifespan is considered as an important root trait (Wang et al 2016), because it determines the quantity of root organic matter transferred to soil (Guo et al 2008) as well as exerting an indirect control on nutrient and water uptake efficiency (Luke McCormack et al 2012). Root turnover varies widely within and among species and across ecosystems (Majdi et al 2005) but a fundamental understanding of the mechanisms that control fine root life span among different climates is poorly understood. Most published studies related root lifespan either to endogenous factors such as root diameter and branch order (Guo et al 2008, Wang et al 2016), seasons of initiation, nitrogen (N) concentration (Luke McCormack et al 2012), root depth (Baddeley & Watson 2005) or to climatic factors such as temperature, water and nutrient availability (Chen & Brassard 2013; Green et al 2005). However, the influence of environmental factors on fine root life span remain limited. Understanding how endogenous factors and environmental factors covary in influencing root longevity is therefore important in order to provide a better understanding of the contribution of fine roots to resource fluxes (Baddeley & Watson 2005).

Agroforestry is a land use management system in which woody perennials (tree, shrub, etc.) are associated with herbaceous plants (agricultural crops, pastures) and/or livestock in a spatial arrangement, a rotation or both (Somarriba 1992). Integrating trees into farming has some obvious advantages in protecting the environment and providing a number of ecosystem services (Jose 2009; Newaj et al 2016), e.g. biodiversity conservation (Harvey et al 2008, Schroth 2004, Schroth & Harvey 2007), soil enrichment (Schroth & Sinclair 2003, Young 1989), water quality enhancement (Anderson et al 2009), increase the amount of carbon sequestered (Nair 2012, Soto-Pinto et al 2009) and land degradation (Garrett et al 2009, Garrity 2004; Nair et al 2009). Tree roots contribute to increase water infiltration and retention in the soil profile by increasing soil porosity and reducing runoff, which reduce moisture stress during drought (Newaj et al 2016). Tree roots in agroforest systems are able to explore a high soil volume for water and nutrients, by developing deeper roots to avoid competition with crop or pasture roots and thus improve soil fertility by root turnover (Chander et al 1998; Lehmann & Zech 1998). However, knowledge about above and below ground relationships in such systems is scanty.

In this study, we examined root elongation, initiation, mortality and survivorship of walnut trees (*Juglans* L.) growing in agroforest systems over 21 months along a climatic gradient in France. We focused on relating root phenology to leaf and stem phenology. We aimed at determining which factor most drives root growth in such systems over different phenological periods. We also characterized the relationship between root and shoot phenology, particularly in response to both temperatures and precipitations. Rhizotrons were used to monitor monthly root growth and root mortality in each site. We hypothesized that (i) shoot and root are asynchronous regardless the climate (ii) the drivers of root growth and root mortality are not the same between climates, (iii) root diameter is linked to elongation rate and is altered over phenological periods and between climates and (iv) root longevity is altered among climates.

2. Materials and methods

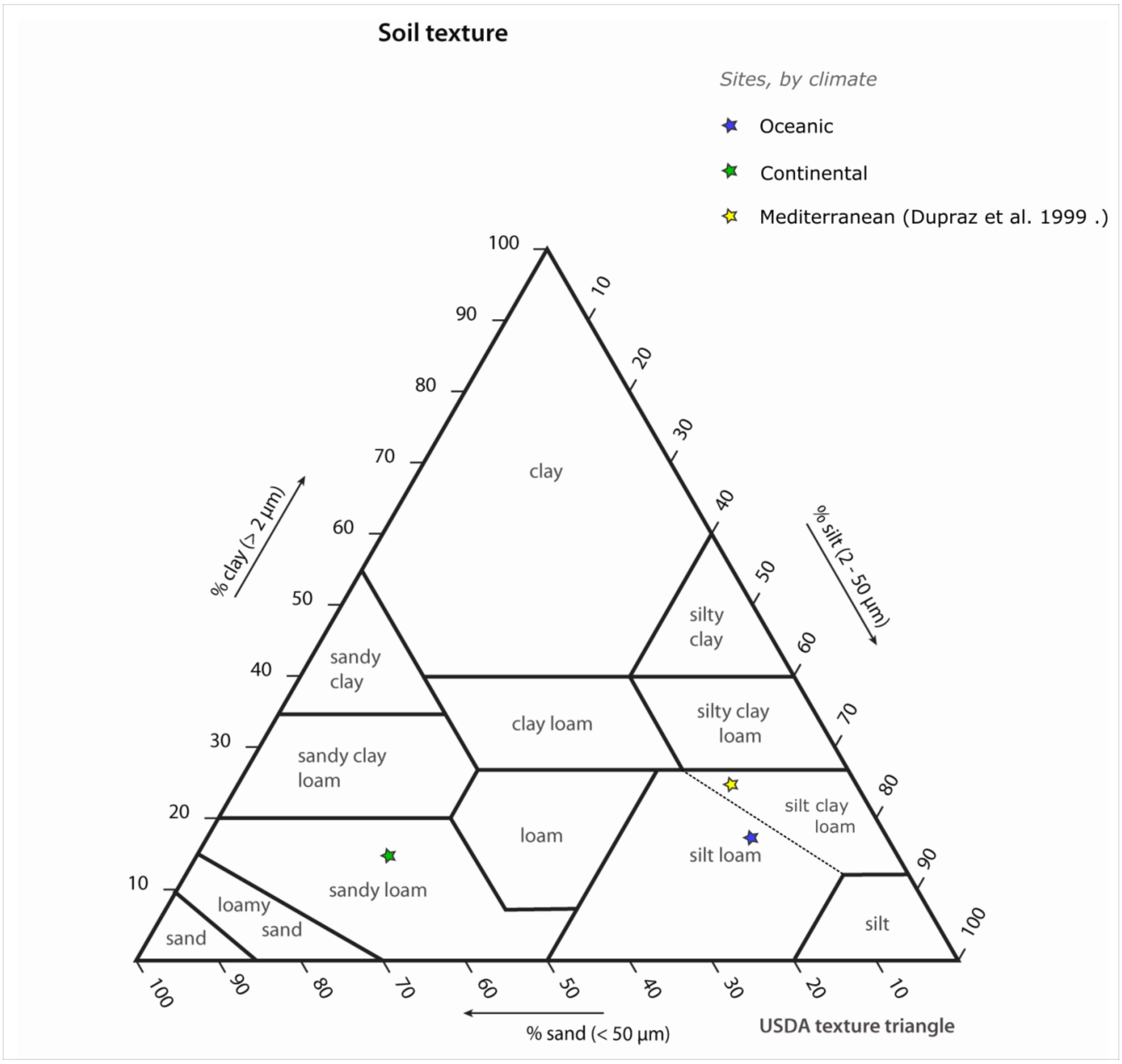
2.1 *Study sites*

Root and shoot phenology of hybrid walnut (*Juglans nigra* × *regia L.*) were studied in three agroforestry systems along a latitudinal gradient in France. Trees were intercropped with pasture or crops (Appendix 1). The most northern site was located at Cormont, Pas de Calais, France (50°33'27.87''N, 1°44'3.08''E, elevation 40 m a.s.l.). Climate was oceanic with a mean annual temperature of 11°C and a mean annual rainfall of 777.9 mm (Météo France) (see section 3.2.2), thus the site is hereafter termed ‘oceanic’ (Appendix 2). The agroforest comprised hybrid walnut (*Juglans nigra* × *regia* L.), common walnut (*Juglans regia* L., 1753), ash (*Fraxinus* L.), sycamore (*Acer pseudoplatanus* L.) and oak (*Quercus* L.) at (13 m x 7.5 m tree spacing) intercropped with permanent pasture (ovine pasture). All trees were planted in 1999 at a total current density of 100 trees ha^{-1}. The soil is silt loam (Table 1; Fig. A) of at least 2.5 m deep, with the presence of the water table at this depth in June. The site is situated next to La Dordonne River. The mean diameter at breast height (DBH) of walnut trees at the site was 0.30 ± 0.03 m and mean height was 14.75 ± 3.50 m.

The second agroforest was located at Le Beil, Madic, Cantal, France (45°22'7.95''N, 2°28'1.46''E, elevation 530 m a.s.l.). Climate was continental with a mean annual temperature of 9.95°C and a mean annual rainfall of 1174 mm (Météo France, thus this site is hereafter termed ‘continental’ (Appendix 2). The agroforest comprised three transplanted tree species: hybrid walnut (*J. major* MJ209 *x J. regia* L.), cherry (*Prunus avium*) and sycamore (*Acer pseudoplatanus*) (at 12 m x 8 m tree spacing), intercropped with permanent pasture (ovine or bovine pasture). All trees were planted in 1994 at a density of 100 trees ha^{-1}. The soil is sandy, particularly acidic, and attained an average maximum depth of 110 cm on a 5° to 10° (Table 1). The site is situated 300 m from Madic Lake. Mean DBH of all walnut cultivars at the site was 0.20 ± 0.02 m and mean height was 12.09 ± 1.30 m. The third agroforest was located at the Restinclières experimental site, 15 km north of Montpellier, Department Hérault, France (43°43’N, 4°01’E, elevation 54 m a.s.l.). The climate is sub-humid Mediterranean with a mean annual temperature of 15.4 °C and a mean annual rainfall of 873 mm (this site is henceforth termed ‘Mediterranean’ (Appendix 2). The study plot comprised hybrid walnut trees (*Juglans nigra × regia* L. cv. NG23) (at 13 × 4 m tree spacing) intercropped with durum wheat (*Triticum turgidum* L. subsp. *durum (Desf.*) Husn). However, rapeseed (*Brassica napus* L.) was also cultivated in 1998, 2001 and 2006, and pea (*Pisum sativum* L.) in 2010 and 2016. All walnut trees were planted in 1995 in lines oriented east west, and the current density is of 110 trees ha^{-1}. The annual crop was fertilized with approximately 150 kg N ha^{-1} yr^{-1} (Cardinael et al 2015) (Cardinael et al 2015). The soil is a silty clay deep alluvial soil (25% clay and 60% silt) (depth > 4m) and slope angle is <1° within the site (Mulia & Dupraz 2006). The site is near the Lez river watershed and the depth from the soil surface to the water table usually oscillates between 5 m in winter and 7 m in the summer (Cardinael et al 2015). More details about the experimental site are available in (Dupraz et al 1999) (Mulia & Dupraz 2006) (Cardinael et al 2015). Mean DBH of all walnut trees at the site was 0.24 ± 0.13 m and mean height was 11.09 ± 2.50 m.

Table1: Soil physical and chemical characteristics

Soil properties												
	Chemical									**Textural (%)**		
	pH	**N (g/Kg)**		**C/N**		**P (g/Kg)**		**K (g/100g)**		**Clay**	**Silt**	**Sand**
	Mean	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	Mean	Mean
oceanic	6,9	0,17	0,1	9,19	1,5	0,15	0,1	1,5	0,1	17,6	66,3	16,1
continental	4,7	0,22	0,1	9,76	1,4	0,04	0	2,82	0,2	15,1	22,6	62,3
Mediterranean	8,4	0,9	0,1	10,7	0,7	-	-	-	-	25	60	15



2.2. *Climatic data*

Global precipitation data for the three sites were obtained from Météo-France (oceanic: Le Touquet, (50°30'48''N, 1°37'18''E, elevation 5 m a.s.l.), (continental: Station Naves, 45°19'12''N, 1°46'18''E, elevation 450 m a.s.l.) and (Mediterranean: Station Restinclières, 43.702 N et 3,86 E, elevation 100 m a.s.l.) respectively. Soil and air temperatures were also measured in all sites in two soil layers: 10 and 60 cm using Thermochron iButtons (DS1921G) (Hubbart et al 2005), Appendix3). The sensors were programed to measure the temperature twice a day (day and night). The device stores the readings for collection and display by a computer via 1-Wire Interface (cable). Soil water potential (noted ψ hereafter) was measured using Irrometers (WaterMark, IRROMETER Company, Inc. USA) installed at each site at depths of 10 and 60 cm (Appendix 4). The WaterMark Monitor automatically reads up to eight sensors and stores the readings for collection and display by a computer via a USB cable. We programed the monitor to measure ψ.

2.3. Rhizotrons installation

To measure walnut root elongation and mortality along the climatic gradient, we installed rhizotrons, also called root windows, in pits at each field site (Appendix 5). Rhizotrons comprised transparent polyvinylchloride (PVC) sheets placed against the soil profile, through which root growth dynamics can be observed (Reich, et al., 1980; Misra, 1999; Mao et al., 2014). In the oceanic site, four trenches (2m long x 1 m wide x 2m deep) were dug in one row of trees and each pit was located at 2 m from the tree trunk. One rhizotron was installed on each opposing face of the trench (n = 8 rhizotrons).

In the continental agroforest (Madic), we dug eight (1 m long x 1 m wide x 1 m depth) trenches in three rows of trees. Each trench was at a distance of 2 m from the nearest tree trunk. Eight rhizotrons (50 cm long x 50 cm wide x 0.5 cm thick), were installed.

At the Mediterranean site, one pit (5 m length x 1.5 m width x 4 m depth) was dug in March 2012 between two walnut trees on the same tree row (Appendix 6) (Cardinael et al. 2015). The pit was reinforced with wooden beams to avoid collapse of the pit walls. In June 2014, two rhizotrons (100 cm long x 80 cm wide x 0.5 cm thick) were installed as well as eight smaller windows (65 cm long x 30 cm wide x 0.5 cm thick) at depths of 20, 115, 220 and 320 cm.

Where the rhizotrons were to be placed on the soil wall, we gently removed the soil to make a flat surface and cut all roots on the profile with secateurs. The soil removed during the digging of the trenches was kept aside, and then sieved through a 5 mm sieve and air-dried for several hours. The sieved and air-dried soil was then poured into the space between the window and the soil profile and slowly compacted using a wooden plank. Rhizotrons were placed vertically against the soil profile at an angle of 15°. This angle will permit the roots to grow downwards due to positive geotropism (Huck & Taylor 1982; Mao et al 2013). Each rhizotron was covered with foil backed felt insulation and black plastic sheeting to protect roots from light and temperature variations. All pits were then covered with wooden boards and corrugated plastic or a metallic roof to avoid damage from passing animals and to prevent direct rainfall and sunlight on the rhizotrons. In the first three months after installation, no root growth was recorded because soil disturbance during rhizotron installation causes over estimations of root growth (Strand et al 2008).

2.4. Measurements of root growth

To measure fine root growth dynamics, we either scanned rhizotrons or used a time-lapse camera to automatically take pictures before then analyzing images (Mohamed et al 2016, submitted). In the continental and Mediterranean sites, a scan of each rhizotron (between two and four images per window depending on the size of the window) was taken at monthly intervals over 21 months using an Epson Perfection V370 flatbed scanner with a high optical resolution of 4800 dpi (Appendix 7).

At the oceanic site, a time-lapse camera (Cuddeback Attack, U.S.A.) was placed on a wooden cleat in front of each rhizotron at a distance of 90 cm from the rhizotron (Appendix 8). Photographs were taken daily at 2 a.m. and 2 p.m for 21 months. The time-lapse cameras ran automatically for several months at a time using alkaline batteries. In September 2014, the trench at the Mediterranean site was flooded due to exceptionally strong rainfall (Appendix 9), therefore all rhizotrons were reinstalled in March 2015. No data were recorded from September 2014 to March 2015, and data were recorded but not used in statistical analysis from March to June 2015 (the three months safety period after rhizotron installation). Similarly, at the oceanic site, nearly all trenches were flooded due to strong rainfall in January 2015 and the proximity of the water table, and almost half the cameras were damaged. In March 2015, all cameras were replaced and no data were recorded from January 2015 to March 2015. To avoid the same problem the following year, we removed cameras from November 2015 to March 2016; therefore data are missing for this period also (Appendix 9).

Monthly measurements of root emergence, mortality and elongation were carried out immediately after roots were observed in each rhizotron (and after the first three months had passed) until October 2014. Roots were classified into three diameter classes: (0-1) mm, (1-2) mm, and (2-5) mm. However for measurements from the camera method, because an overestimation of diameters (Mohamed et al., 2016 submitted), we corrected the value of roots diameter by using the following equation:

$y= 0.43x$

Where y represents the value corrected of root diameter, x represents the real value of the diameter taken by the camera. 0.43 is the relative value corresponding to the mean gap between scanner measurement and camera measurement. We also classified root topology onto two orders: ‘parent’ or ‘child’ (SmartRoot software). The effect of Root topology orders at the oceanic site was excluded from the analysis because very few lateral roots emerged.

In previous studies, root mortality was assumed to occur when the root became darker in color (West et al 2004) or when it disappeared (Baddeley & Watson 2005). In some studies, both criteria were applied (Wells & Eissenstat 2001) (Anderson et al 2009). In our study, color was the only criterion applied (Mao et al 2013a), as root death can occur before its disappearance (Hooker et al., 2000). We declared the root dead when it was turning black with no growth between two or more successive sessions until the last observation date occurred.

2.5. *Image analysis*

Once images of root growth had been acquired, we conducted analysed images using the semi-automated SmartRoot software (Lobet et al 2011) (Appendix 10). SmartRoot is an operating system independent freeware based on ImageJ and using cross-platform standards (RSML, SQL, and Java) for communication with data analysis softwares (Lobet et al 2011) (Mathieu et al 2015). Before analyzing roots, it was necessary to stitch images together (e.g. with Adobe Photoshop CS3 software) if several have been taken for the same rhizotron (because the rhizotron surface area was greater than the field of the scanner). We transformed all images to 8 bit gray scale and then inverted them using ImageJ software so that roots were darker than the background of the image. The length and diameter of each root produced during one interval time (i.e. one month) were calculated for each rhizotron. Before analyzing a new sequence of images, SmartRoot provides the user with an icon to import the previous data file on this new image, which helps the estimation of the evolution of root length. This preceding image also helps determine whether the root is live (usually cream in color) or dead (turning black) (Huck & Taylor 1982) (Mao et al 2013b).

2.6. Aboveground phenology and stem growth

We assessed the timing of shoot production through changes in the phenological phases of leaves throughout the year. We divided the year into three phenological periods, early growing season (from budburst to 100% leafing out of early green leaves), late growing season (100% leafing out to leaf-fall) and dormancy (leaf-fall to budburst) (Appendix 11). Shoot phenological periods were recorded using a time-lapse camera (Wingscapes TimelapseCam8.0) at a resolution of 5 megapixels in the oceanic and continental agroforests. One camera per site was placed on the trunk of one tree facing the line of Walnut trees. Photographs were taken daily at 12 a.m. and 12 p.m. At the Mediterranean site, shoot phenological periods were recorded visually using binoculars.

We also measured the radial growth of tree trunks by installing dendrometers Increment Sensor DB20) on eight trees per site. The dendrometers were girth bands, comprising a steel band, spring and nonius scale. Bands were placed on the stem, at a height of 1.3 m. Measured values are read at the nonius scale with 0.1 mm precision. Readings were made monthly at both continental and Mediterranean sites. However, readings were made every three months at oceanic site. Tree height was recorded annually after leaf shedding using a hypsometer Vertex (Quebec, Canada).

2.7. ***Root growth and mortality dynamics***

We used the following methods to estimate root elongation rate, initiation and mortality:

(i). Individual root growth was evaluated by calculating the difference between the root length at initiation t_{-1} and at the day on which the measurement was made t To determine the daily root elongation rate (RER), the mean of all individual root lengths produced between time t and t_{-1} was divided by the duration of the corresponding period.

$$RER_{t\text{-}1,\,t} = \frac{len._{t} - len._{t\text{-}1}}{P_{t\text{-}t\text{-}1}}$$

Where, $RER_{t-1,t}$ is the daily RER for all roots undergoing growth (zero values were excluded); $len._{t-1}$ and $len._{t}$ are the lengths of the root n at inventory time t_{-1} and t, respectively; $p_{t-1,t}$ is the period between inventory time t_{-1} and t (d).

(ii). Monthly mean root initiation quantity (RIQ), was calculated as the mean number of new roots initiated between time t and $t_{-1.}$

(iii). Monthly mean root mortality quantity (RMQ) was calculated as the mean number of dead roots between t and t_{-1} only when alive roots are presents.

2.8. *Statistical analysis*

All root dynamics indicators including mean root elongation rate (RER), mean root initiation quantity (mRIQ), mean root mortality quantity (mRMQ) and root survivorships were calculated using R software.Version 2.15.3 (R Development Core Team 2013). RER for growing roots only was performed in the statistical test. Statistical test was performed for mRMQ when the total number of alive roots was > 0. A Shapiro-Wilk test was performed before each test to ensure if the investigated indicator followed a normal distribution and homogeneity of variances was checked. For data not normally distributed, analyses were performed by a Kruskal-Wallis Test. Effects of season, site, diameter classes and topology orders were tested on each of roots indicators. A post-hoc analysis was performed when Kruskal-Wallis result was significant using Nemenyi test of Kruskal Wallis at $p<0.05$ to determine which levels of the independent variable differ from each other level. Spearman's rank correlations (Spearman) were performed to determine the relationship between the climatic variables (mean soil and air temperatures and mean ψ) and the mean RER, mRIQ and mRMQ.

A Cox Proportional Hazard Model was used to analyze the effects of different varying factors on root mortality risk. Factors used as variables included sites, phenological period, diameter classes and topology orders. Different Cox Hazard Models were done for two roots topology orders: parent and child order (SmartRoot software). We performed the statistical test when only the sample size was >20 for each category (topology order, diameter classes, sites, periods). All analyses were performed using R software, Version 2.15.3 (R Development Core Team 2013) at a significance level of <0.05.

3. Results

3.1 Latitudinal gradient of meteorological and soil hydrological data

Mean annual temperature in over the two year period was 11.7°C, 12.4°C and 14.8°C at the oceanic, continental and Mediterranean climates, respectively. Mean monthly air temperature over the same period was lowest in December at all field sites (ranging from 3.1°C to 7.6°C; Fig. 1), and highest in July at the oceanic (17.8°C), continental (20.6°C) and Mediterranean site (24.3°C) (Fig. 1). Average annual precipitation over 2014 and 2015 was 910 mm, 1056 and 938 mm at the oceanic, continental and Mediterranean sites, respectively (Fig.1). Rainfall at the Mediterranean site was highly variable between the two years, with 1264 mm in 2014 and only 613 mm in 2015 (Fig. 1). A negative and significant ($p<0.001$, $r= -0.40$) correlation was found between total annual rainfall and ψ.

Soil water potential was significantly higher at the Mediterranean site than those of continental and oceanic sites ($p<000.1$) and the oceanic site had a significantly higher ψ than continental site ($p<0.0001$).

Soil and air temperatures at the Mediterranean site were both significantly higher than those of continental and oceanic sites ($p<0.0001$) and the oceanic site had a significantly greater soil temperature compared to the continental site (p=0.04). However, no significant differences were found in air temperature between oceanic and continental sites (p=0.17).

3.2 Leaf phenology

The date of budburst differed among sites: the Mediterranean trees in 2015 had an earlier budburst (18 April 2015) than the continental and oceanic climates by 15 days and 27 days, respectively. In 2016, this difference increased to 21 and 28 days, respectively (table1). The Mediterranean trees had an earlier early green leaf unfolding date (2 June 2015) than the continental and oceanic climates by 3 and 16 days, respectively. Mediterranean trees had later leaf fall (18 November 2014 and 12 November 2015) than the continental (6 days in 2014 and 15 days in 2015) and oceanic sites (10 days in 2014 and 9 days in 2015).

Mean soil temperatures during the early growing season at the oceanic site was 12.7°C, compared to 14.1°C and 11.9°C at the continental and Mediterranean sites, respectively, while reached (13.9°C, 15.8°C, 17.9°C) during LGS and (9.6°C, 10.8°C, 6.7°C) during the dormant season at oceanic, continental and Mediterranean sites, respectively.

3.3 Stem phenology

The timing of root growth was synchronous with that of stem growth during late growing season (from June to November) in both study sites (Fig. 3). Stem growth started when trees had 100 % of green leaf unfolding (the onset of late growing season). Root and stem growth had an antagonistic growth pattern (Fig.3), i.e. peaks of root growth occurred inversely to those of stem growth. Stem growth then was dropped down sharply with the leaf fall. The length of stem growing season was shorter than that of roots by 86 days and 73 days at both Continental and Mediterranean sites, respectively.

3.4 Root elongation rate (RER)

Mean RER for growing roots at all sites was influenced significantly by season, with one distinct flush of root growth observed during the aerial growing season (Fig.2), and negligible growth during the rest of the year. Peaks of mean RER always lagged behind those for budburst. In 2015, mean RER was initiated before budburst in both oceanic (28 days) and continental (26 days) climates. However at the Mediterranean site, budburst preceded root elongation by 28 days in 2015. Mean RER peaked in June (Mediterranean) or July (oceanic and continental; Fig. 2), before decreasing and then peaking to a lesser extent in August for the Mediterranean site and September for the oceanic and continental sites (Fig. 2). When mean RER (during all observation periods) between all sites was compared, mean RER was significantly higher (df=2, $p = 0.01$) at the oceanic site compared to the continental site only.

No significant relationships were found between RER and root diameter classes. No significant differences were found in RER of any roots between the early and dormant seasons.

When all root data were combined, mean RER was positively and significantly correlated with the mean air and soil temperatures of the month preceding the RER measurement at the oceanic ($P<0001$, $r=0.55$) and continental ($P<0001$, $r=0.48$) sites only (Fig.4a,b). Mean RER was correlated with mean ψ at oceanic site only (Fig.4c, $P<0.001$, $r=0.55$). However, when mean RER of roots growing during the aerial growing season were examined, positive and significant correlations were found between mean RER and soil temperature (Fig.5a, $p=0.002$, $r=0.61$) and mean RER and air temperature (Fig.5b, $p=0.004$, $r=0.48$) of the month preceding growth at the oceanic site only. The mean RER was significantly correlated with mean ψ in both oceanic (negatively) (Fig.5c, $p=0.004$, $r= -0.48$) and Mediterranean (positively) (Fig.5c, $p=0.05$, $r= 0.50$) sites only.

3.5 Comparing and above and belowground phenology

Phenological periods of growth differed between sites. In 2015, the Mediterranean climate had a longer aerial growing season (208 days) than continental and oceanic climates by 30 days and 36 days, respectively. In 2014, the dormant season was shorter at the Mediterranean site (151 days) compared to continental (172 days) and oceanic sites (188 days). However, in 2015, the length of the growing season was similar in oceanic and Mediterranean climates but the dormant season was longer by 36 days at the continental site (Table.1). The duration of the growing season of roots was longer than that for leaves for the oceanic (38 days), continental (62 days) and Mediterranean (8 days) sites (Table.1). Root growth was not synchronous with leaf phenology in any climate or either year. However, root elongation was synchronous with trunk growth in the three climates (Fig.2).

3.6 Mean monthly root initiation quantity (mRIQ) and mean monthly mortality quantity (mRMQ)

The mean root initiation quantity (mRIQ) was always highest during the late growing season compared to the other seasons. The first peak of root appearance at each site was 3 months after rhizotron installation (October 2014 at both the oceanic and continental sites and in June 2015 at the Mediterranean site). In 2015, peaks of mean root initiation were found in June at the oceanic site (1.17± 2.32 roots), in July at the continental site (3.6 ± 6.02 roots) and in October at Mediterranean site (1.71±3.29 roots; Fig.5). Each peak of mRIQ was followed immediately by a peak of mRMQ in all climates during the late growing season (Fig.5). A significantly lower number of roots was initiated (p=0.02) at the oceanic site (0.66± 1.87 roots) compared to both continental (0.7± 5.3 roots) and Mediterranean climates (1.9±4.9 roots). No significant differences in mRIQ were found between continental and Mediterranean sites.

No significant differences in mRMQ were found between climtes. No significant differences in mRMQ were found between late growing and dormant seasons, or between early and late growing seasons. mRMQ was significantly higher in the late growing season compared to the dormant season ($p = 0.009$).

During the late growing season, mRIQ was not correlated with mean ψ in any of the three climates (Fig.7c). Significant negative correlations were found between mRIQ and mean soil temperature of the preceding month at the continental ($P< 0.0001$, $r= -0.28$) and Mediterranean ($P< 0.0001$, $r= -0.54$) sites only (Fig.7a). Mean air temperature of the preceding month was negatively correlated ($p=0.0002$, $r=-0.37$) with mRIQ in Mediterranean climate only (Fig.7b). However, mRMQ increased rapidly with the increase of mean soil temperature and mean ψ and peaked when ψ was maximal at continental site only (Fig.8c). Whereas, mRMQ was not correlated with mean soil or air temperatures or mean ψ in both oceanic and Mediterranean sites (Fig.8a&b). When all factors were considered together, mRIQ and mRMQ of first order roots (parent) was significantly greater than lateral order (child) at continental site only ($p<0.0001$).

Table 1. The length of the growing season for both shoot and roots in the three climates

Phenological growth periods	**EGS (days) Early growing season**		**LGS (days) Late growing season**	**DS (days) Dormant season**		**Total GS (days) growing season**
Shoot						
Year	2015	2016	2015	2014-2015	2015-2016	2015
Oceanic						
Date	15 May	12 May	18 Jun	08 Nov	03 Nov	
Length	34	-	138	188	191	172
Continental						
Date	3 May	5 May	05 Jun	12 Nov	28 Oct	
Length	33	36	145	172	190	178
Mediterranean						
Date	18 Apr	14 Apr	02 Jun	18 Nov	12 Nov	
Length	45	-	163	151	154	208
Root						
Oceanic	210			100	154	210
Continental	240			118	124	240
Mediterranean	216			-	126	216

- is no observation recorded

3.7 Root survivorship

Cox's proportional hazards regressions showed that when all root diameter classes were grouped together, mean root longevity at the continental site was significantly greater than for roots at the oceanic(z=7.7, $p<0.001$) and Mediterranean (z=14.2, $p<0.001$) sites. The longevity of roots at the Mediterranean site was significantly shorter than at the oceanic site (Fig. 9a, Table2). Compared to the continental climate, the risk of mortality was 2.7 times greater for roots from the Mediterranean site and 2.1 times greater for roots from the oceanic site. Phenological period had an important effect on root longevity at continental site but not at oceanic and Mediterranean sites during the observation period. At the continental site, compared to the dormant season, the risk of mortality was 1.8 times more during the early growing season and 1.7 times more during the late growing season (Fig. 9b). No significant differences in longevity were found between growing seasons at the other two sites. Root diameter classes had the largest effect on root longevity compared with other factors. When lateral roots were excluded from the analysis (for all growth periods combined), roots in the 0.1 mm diameter class lived for significantly shorter periods compared to those in the 2-5 mm diameter class at continental (z= -1.65, p=0.006) and Mediterranean (z= -3.36, p=0.0007) sites only. However, no significant differences in longevity was found between roots from the 0-1 mm and 1-2 mm diameter classes (Fig. 9c). At the continental site, roots in the 2-5 mm diameter class had 38% less risk of mortality than roots in the 0-1 mm diameter class. At the Mediterranean site, roots in the 2-5 mm diameter class had 61% less risk of mortality than those from 0-1 diameter class. When all factors were considered together, except topological order, first order roots lived longer than lateral roots at the Mediterranean site (z= -3, p=0.005) but not at the continental site. At the Mediterranean site, first order roots in the 0-1 mm diameter class had 44.5 % less risk of mortality (z= -3.04, p= 0.002) than that of lateral roots for the same class of diameter, but no differences in longevity were found at the continental site. First order roots in the 1-2 mm diameter class significantly had longer longevity (z= -2.7, p=0.005) than lateral roots of the same class of diameter at Mediterranean climate, but not at the continental site.

4. Discussion

We did not find any significant differences between climates with regard to the phenology of root dynamics throughout the year. As also found by (Contador et al 2015) in Mediterranean climate, walnut hybrids had one marked distinct flush of root growth during the aerial growing season in all three climates with much less root growth during the aerial dormant season.

Temperature and soil water potential effects on root growth dynamics

Mean RER of walnut trees was positively correlated with both mean soil and air temperatures at the oceanic and continental sites only. It is surprising that we did not find any relationships between mean RER and temperature at the Mediterranean site, as Germon et al (2016) found a highly significant and positive correlation between RER and soil temperature for the same stand of walnut cultivars. However, mean RER was significantly and positively correlated with mean air and soil temperatures during the late growing season, where the highest peaks of root growth were found at the oceanic site only. Mean RER at the oceanic site was also significantly and negatively correlated with mean soil ψ of the preceding month, i.e. as was also found by Joslin et al., (2001) for "oak (*Quercus prinus* L.) and white oak (*Quercus alba* L.) growing in a subtropical climate. However, mean RER at the Mediterranean site was significantly but positively correlated with mean soil ψ, similar to results for (*Abies balsamea* L.) (Olesinski et al 2011) and for (*Quercus alba* L.) seedlings (Reich et al 1980). At the continental site absence of correlations of RER with any climatic factors is in conflict with other studies which indicate that air and soil temperatures are the prominent factors driving RER (Misra 1999), (Hendricks et al 2006) (Mao et al 2013b) (McCormack & Guo 2014) (Germon et al 2016) (Gill & Jackson 2000) especially during the growing season in temperate climates. In our study, for all climates, soil temperature never reached below 3°C or above 21°C during the entire study period. Most root elongation occurred when the soil temperature was within the range 9 - 17°C. Our results are contradictory to those found (Heninger & White 1974, Lyr 1996) who showed that the highest rates of root growth of deciduous trees under non limiting moisture conditions were usually found when soil temperatures were above 20°C. Here we found that the highest rate of elongation occurred when temperatures were between 14 - 17°C. At the oceanic site, soil temperature varied little throughout the year, with few extreme values, whereas the continental site had large seasonal differences in soil temperature. Mean soil ψ at the Mediterranean site was significantly lower than at the oceanic and continental sites. Soil ψ can limit root elongation by either excessive water resulting in anaerobic conditions or inadequate water to support growth (Joslin et al 2001). Previous studies have shown that walnut seedlings have a low resistance to water stress and are sensitive to waterlogging both between and within cultivars (Mapelli et al 1995), phenomenon that we confirmed here (waterlogging in Oceanic site and water stress in Mediterranean site).

Our results demonstrated a decline in RER with the decrease of mean soil ψ at the Mediterranean site, suggesting that soil water is limiting for root growth in the superficial layers, as found for other broadleaf species (e.g. Wan et al., 2002 studying *Gutierrezia sarothrae* subshrub). However, at the oceanic site, our results showed that RER augmented with the decrease of mean soil ψ and declined with the increase of mean air and soil temperatures, also found by Joslin et al, (2001) (Joslin et al 2001) for (*Quercus prinus* L.). Our results indicate that at the oceanic site, soil temperature played a major role in driving root elongation, as also found by (Germon et al 2016) (Mao et al 2013b) (McCormack & Guo 2014) and that soil ψ played an indirect role, but as both factors co-vary during the late growing season, it is difficult to separate their distinct effects on root growth.

Our results suggest that, once soil temperature is favorable for roots, and if there are no extreme of temperature throughout the year, then other limiting factors will drive root growth. For example, root growth decreased substantially irrespective of soil temperature during a period of drought (Reich et al, 1980) (Reich et al 1980) (e.g. Mediterranean site in our study), and will increase positively with the increase of both ψ and soil temperature when there was a saturated soil, depending on the factors limited which change among species and their tolerances to stress conditions. Surprisingly and contrary to the observations of previous authors (e.g. Germon et al, 2016 working on walnut cultivars, Mao et al, 2013 studying *Picea abies* and *Abies alba* and (Kern et al 2004) studying *Populus deltoides Bartr*), mean RER was not related to root diameter. While trees growing at the continental site produced many short-lived lateral roots, no lateral root initiation occurred at the Mediterranean and oceanic sites. Soil conditions could also have played a role in this lower RER associated with higher lateral roots development at continental site. A possible reason is a strong soil acidity that has been showed to increase heavy metal solubility and development of lateral roots in consequences (Kahle 1993). Moreover, the lower phosphorus content at the continental site is also a factor already found to influence root architecture and lateral development, in a purpose to increase soil exploration (Rao et al. 2015).

Mean RIQ and mRMQ were related to annual variations in soil temperature, except for the first peak of root initiation which occurred three months after the rhizotron installation at the three sites regardless of the phenological period (Johnson 2001) (Baddeley & Watson 2005) . We consider this result as an artefact of the rhizotron method, which led to an overestimation of the fine root production (Hendrick & Pregitzer 1996a) (Majdi 1996) (Majdi et al 2005) (Green et al 2005) (Metcalfe et al 2008). The second peak of mRIQ was found during the late growing season, regardless of climate, and was followed immediately by a peak in mRMQ. Therefore, the major pulse of hybrid walnut root production is inherently programmed to occur during the late growing season (June-Novomber) with significantly less production in the aerial dormant season, as also found in many deciduous tree species in temperate zones (e.g., Joslin 2000; Hendrick & Pregitzer, 1996) (Hendrick & Pregitzer 1996b) (Joslin et al 2000). Psarras et al, (2000), also found a peak of root emergence *in Malus sylvestris* (L.) Millin late June and early July, which coincided partially with major phases of shoot and fruit growth.

In our study, mRIQ was significantly higher at the continental site compared to the two other sites during the late growing season. A first possible reason is to link to the phenomenon responsible of higher development of lateral roots. Another explanation for this greater productivity is that soil and air temperatures and ψ were optimal for growth during the late growing season. Mean RIQ was correlated with mean soil temperature at the Mediterranean and continental sites only, as was expected (Comas et al, 2005) (Mao et al 2013a), but the lack of significant relationships at the oceanic site is not understood. However, air temperature at the three sites was correlated with mRIQ during the late growing season. These results are in consistent with the observations of (Radville et al 2016a), (Fukuzawa et al 2013) 29] and (Steinaker et al 2010), who demonstrated that temperature was a main driver of root initiation in temperate environments. Tierney et al, 2003 also showed that mean fine root production of sugar maple trees was strongly associated with mean air temperature but not soil moisture or nutrient availability. However, in a literature review (Abramoff & Finzi 2015) suggested that for Mediterranean trees, endogenous factors and the allocation of stored carbohydrates were dominant drivers of root growth.

We found that mRMQ did not differ significantly between sites and was highest during the late growing season. These results are contrary to the finding of Kern et al, (2004), working on *Populus deltoides Bartr.* in a continental climate, who found that mortality was greatest after the end of the growing season. mRMQ was correlated with both mean soil temperature and mean ψ at the continental climate only. Our results are in agreement with the finding of (Harris et al 1995) studying *Acer saccharum* in a moderate continental climate, that root mortality increased in warmer soil temperatures. We consider the peak of mortality as a consequence of a trade-off between competing plant sinks to balance carbohydrate availability. These results suggest that, if other factors are equal, the growth of new roots and the death of existing roots are accelerated with the increase of soil temperature.

Above and belowground phenological relationships

The timing of root growth was asynchronous with that of budburst at all sites, and the spring root flush occurred several weeks after budburst. As both budburst and root emergence are very sensitive to local temperatures (Du & Fang 2014) (Tierney & Fahey 2002), a rapid increase in air temperature in April/May would stimulate budburst quickly. Soil is buffered against rapid changes in air temperature, therefore the subsequent cambial activity in roots would take longer to occur, and root flushes will usually occur after bud burst (Pregitzer et al 2000). Maximal root and radial stem growth both took place during the late growing season. Peaks of stem and root radial growth at the Mediterranean site occurred later in the season (September) than at both other sites (July), possibly linked to precipitation events after the hot, dry summer.

Our results suggest a trade-off between competing plant sinks (Radville et al 2016a). For example, fine root growth was likely fueled by non-structural carbohydrates (NSC) stored before the onset of the aerial growing season, as suggested by Gaudinski et al, (2009) and Najar et al (2014). The decrease in fine root elongation observed in August (oceanic and continental sites) and July (Mediterranean site) may be due to NSC being used for radial growth and fruit production. NSC production from photosynthesis would then increase during the summer, fueling a second root flush, before leaf senescence in November. The decrease in photosynthetic rates at the end of the growing season would result in less NSC being available for radial growth, which decreases rapidly in September – October (Radville et al 2016a) (Du & Fang 2014) (Abramoff & Finzi 2015). Minor root elongation can occur during aerial dormancy at all sites, using local NSC stocks as energy for growth.

Root survivorships

We showed that root longevity differed significantly between climates and roots lived longest at the continental site. Fine roots at this site were significantly thicker and root diameter was correlated to longevity, as also shown by e.g., Anderson et al, (2003) and Wells and Eissenstat, (2001). Roots in the 0-1 mm and 1-2 mm diameter classes lived for significantly shorter periods compared to those in the 2-5 mm diameter class at continental and Mediterranean sites only. Thicker roots have lower N concentration, lower surface area and higher C content than finer roots and thus longevity is increased because of a decrease in metabolic activity (Luke McCormack et al 2012) (Guo et al 2008, Guo et al 2004) (Baddeley & Watson 2005).

As root traits may be prominent drivers of ecosystem processes (McCormack et al, 2015), and as root topological order can influence traits, considering root topology when studying root survivorship has become fundamental (Guo et al, 2008). In our study, first order roots lived longer than lateral roots at the Mediterranean site only and first order roots in the both 0-1 mm and 1-2 mm diameter classes had 44.5% less risk of mortality than that of lateral roots for the same class of diameter, as also found by (Luke McCormack et al 2012). Guo et al, (2008) also showed in longleaf pine (*Pinus palustris*. Mill.) that higher order roots had 46% greater longevity than roots one order lower. We suppose that first order roots live longer than lateral roots because of the greater resource investment in their construction. Finer lateral roots cost less to construct, and so can grow quickly in case of need for soil exploration for limiting resources.

The risk of root mortality at the continental site was significantly greater during the growing season compared to the aerial dormant season, as also found in apple (*Malus sylvestris.* L) (Psarras et al 2000). However, our results are contrary to those of Wang et al, (2016) (Wang et al 2016), who found that the mortality hazard ratio of *Picea abies* and *Abies alba* initiated in the late growing season was reduced by 26.8% compared to roots that emerged in the early growing season. Root longevity usually decreases with increasing temperature (King et al 1999) (Majdi et al 2005), therefore, as temperature fluctuations were more extreme at the continental site, roots may die more quickly as summer temperatures increase rapidly. As root density was higher during growing season at the continental site, soil herbivores and pathogens may be more active (Guo et al 2008).

5. Conclusion

Root studies progressively increase, but it still difficult to draw any firm conclusions about how global changes factors will affect root dynamics or how changes in root dynamics might affect plant production or carbon cycling in soil. The main reason may be the difficulty to generalize this impact in the face of broad variability in responses among plant species, biomes and climates, as well as the variability introduced by methodology (Norby and Jackson, 2000).

In this study, we compared fine root phenology (root elongation, initiation, mortality and survivorship) in relationship with shoot phenology (leaf phenology and stem growth) of walnut trees across three temperate agroforest systems during different phenological periods over the year. Our results showed that, factors driving root production and mortality were not the same neither between climates nor within climate. Our results highlight that, once soil temperature is favorable for roots, and if there are no extremes of temperature throughout the year, then soil temperature is not the main driver of root growth and other limiting factors will drive root growth such as soil water availability (e.g. Mediterranean site in our study). As well as for root occurrence and mortality, if other factors are equal, the growth of new roots and the death of existing roots are accelerated with the increase of soil temperature (e.g. continental and oceanic sites in our study). We showed also clear differences between shoot and root phenology and the length of both aerial and belowground growing season within climate and between climates. Here, as the major pulse of hybrid walnut root production is inherently programmed to occur during the late growing season (5-6 months), with significantly less production in the aerial dormant season in the three climates, our results suggest that the length of both aerial (6-7.5 months) and belowground (7.5-8.5 months) growing season did not drive neither the timing of root production nor the amount of root production. Contrary to the theory that in warmer climate, an earlier onset and a longer growing season period may accelerate forest growth and more C uptake if other factors are not limiting (Du and Fang, 2014). Moreover, through a multi-covariate analysis of root survivorships (site, root diameter, root topology orders and phenological periods), we found that all these covariates were positively correlated with root survivorship. Among these factors, the effects of site and root diameter were the strongest predictor to root survivorship. In our study, we thus showed the influence of different climatic factors on root and shoot phenology along a latitudinal gradient. Our results call for further analyses on the role of site conditions (altitude, topography, plant genotype) in determining tree responses to climate change. An interesting next step will be to focus on better understanding how edaphic and climatic factors interact in natural environments to influence the fine root phenology of plants at various temporal and spatial scales. In addition, the seasonal phenology of trees is a main driver of C allocation from shoots to roots, thus further research is also required to evaluate more precisely the relationship between the internal dynamics of tree carbon and nutrient resources and root phenology.

6. Abbreviations

RER: Root elongation rate, RMQ: Root mortality quantity, RIQ: root initiation quantity, EGS: early growing season, LGS: late growing season, DS: dormant season.

7. Figures

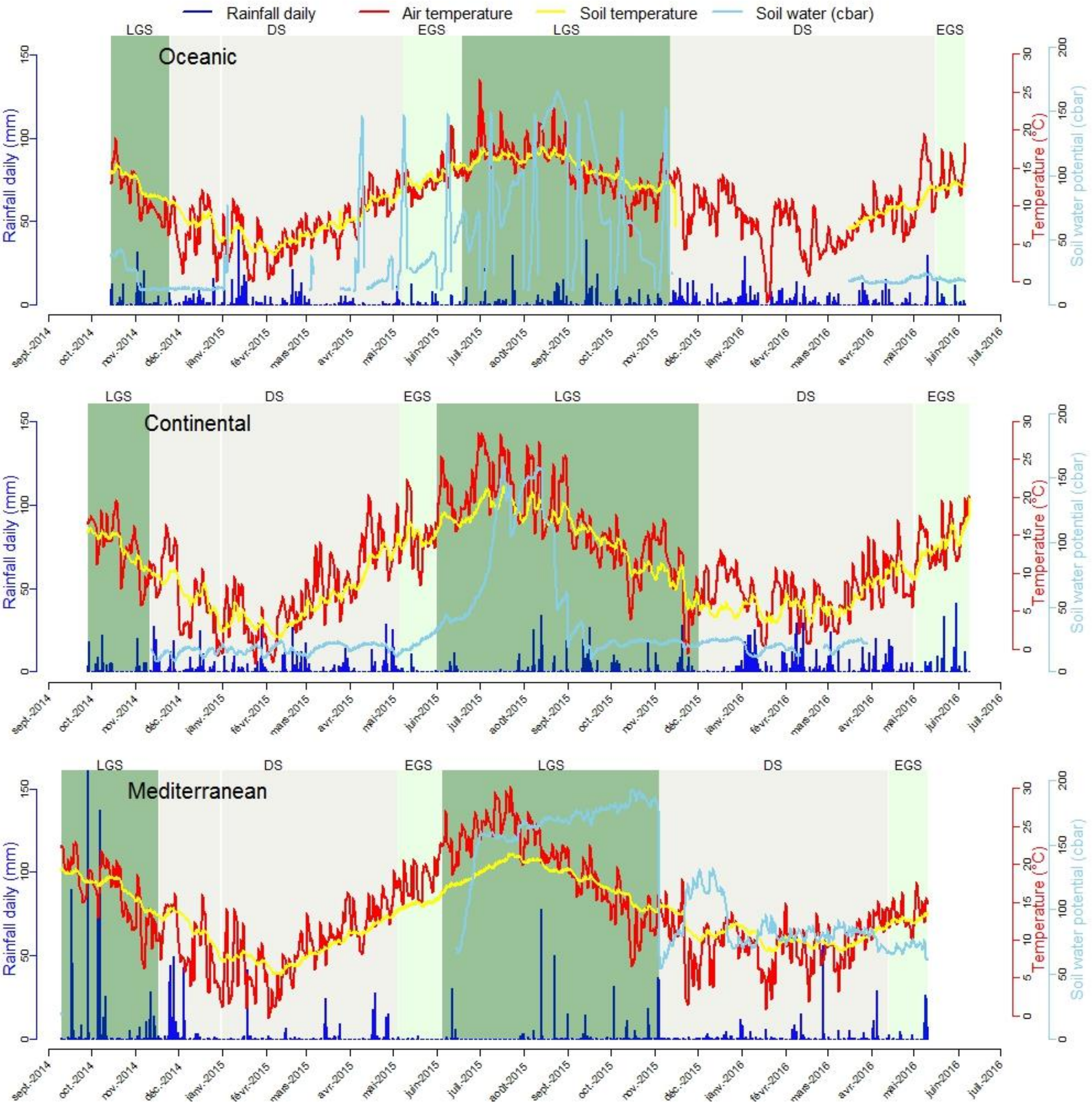


Fig 1. Soil (yellow line) and air (red line; measured at a height of 1.5 m above rhizotrons) temperatures, daily precipitation (blue bars) and soil water potential (light blue line) at a) oceanic, b) continental and c) Mediterranean field sites over the observation period from September 2014 to June 2016. Different background colors correspond to different phenological periods: “LGS” is late growing season (green) “EGS” is early growing season (light green), “DS” is dormant season (gray).

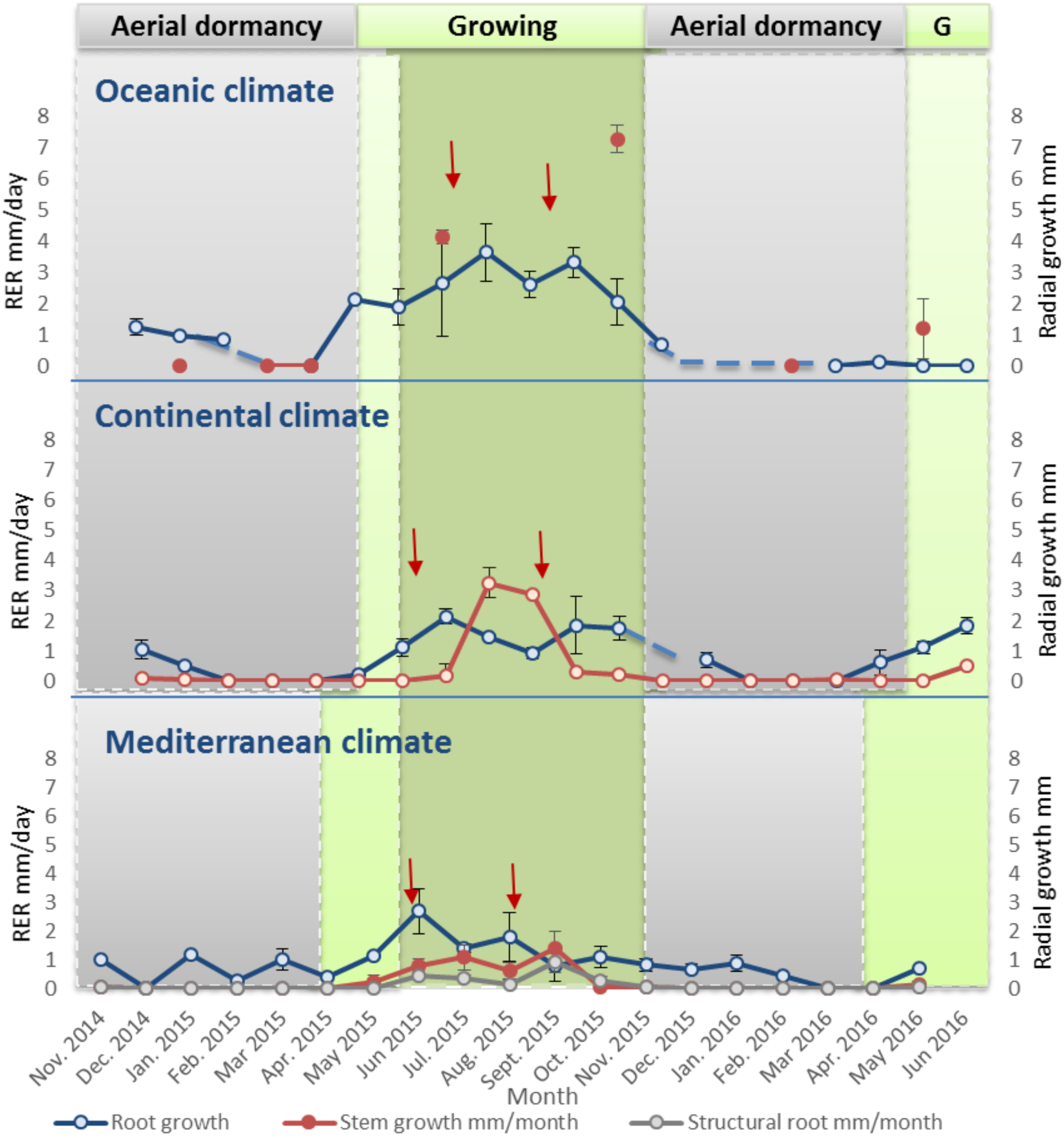


Fig 2. Mean root elongation rate (RER, blue line) and trunk radial growth (red line) at a) oceanic, b) continental and c) Mediterranean field sites over the observation period from September 2014 to June 2016. Different background colors correspond to different phenological periods: “LGS” is late growing season (green) “EGS” is early growing season (light green), “DS” is dormant season (gray). Missing data/ flood damage is shown with a dotted line over the dashed curves.

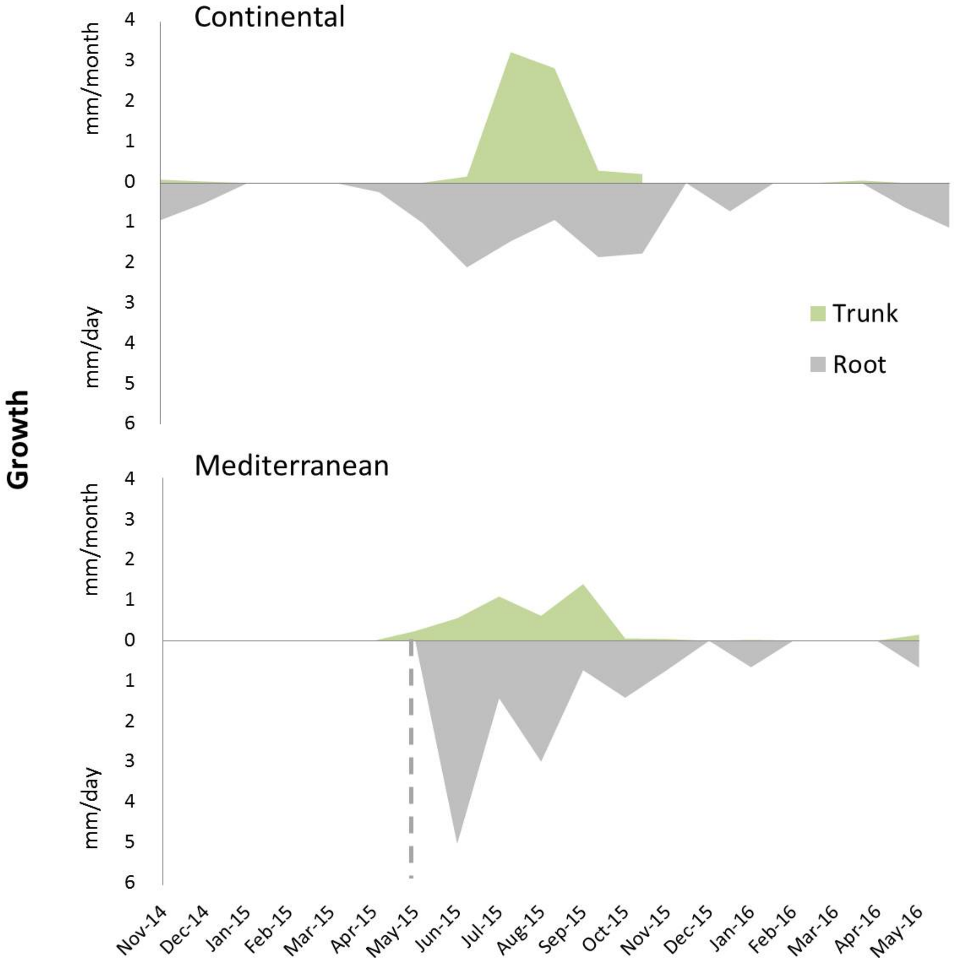
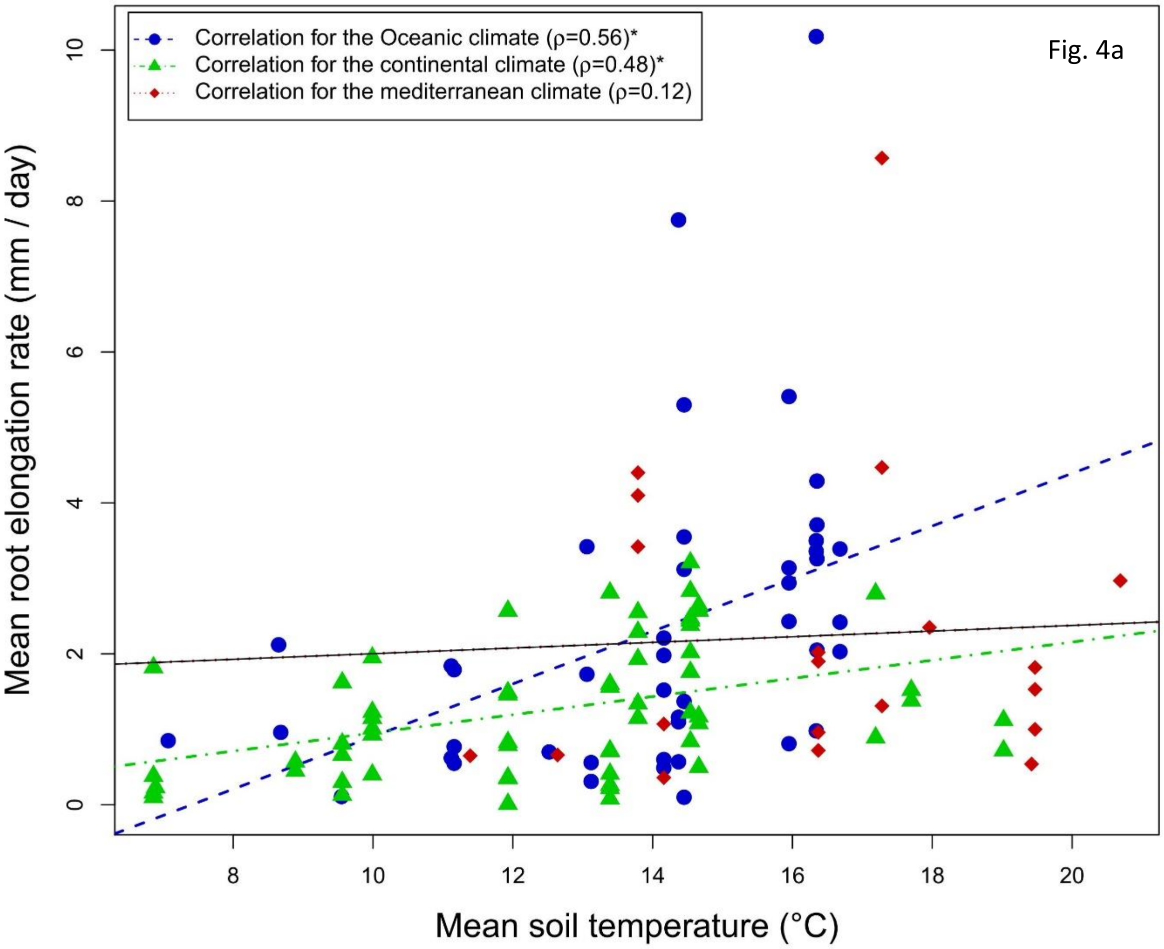
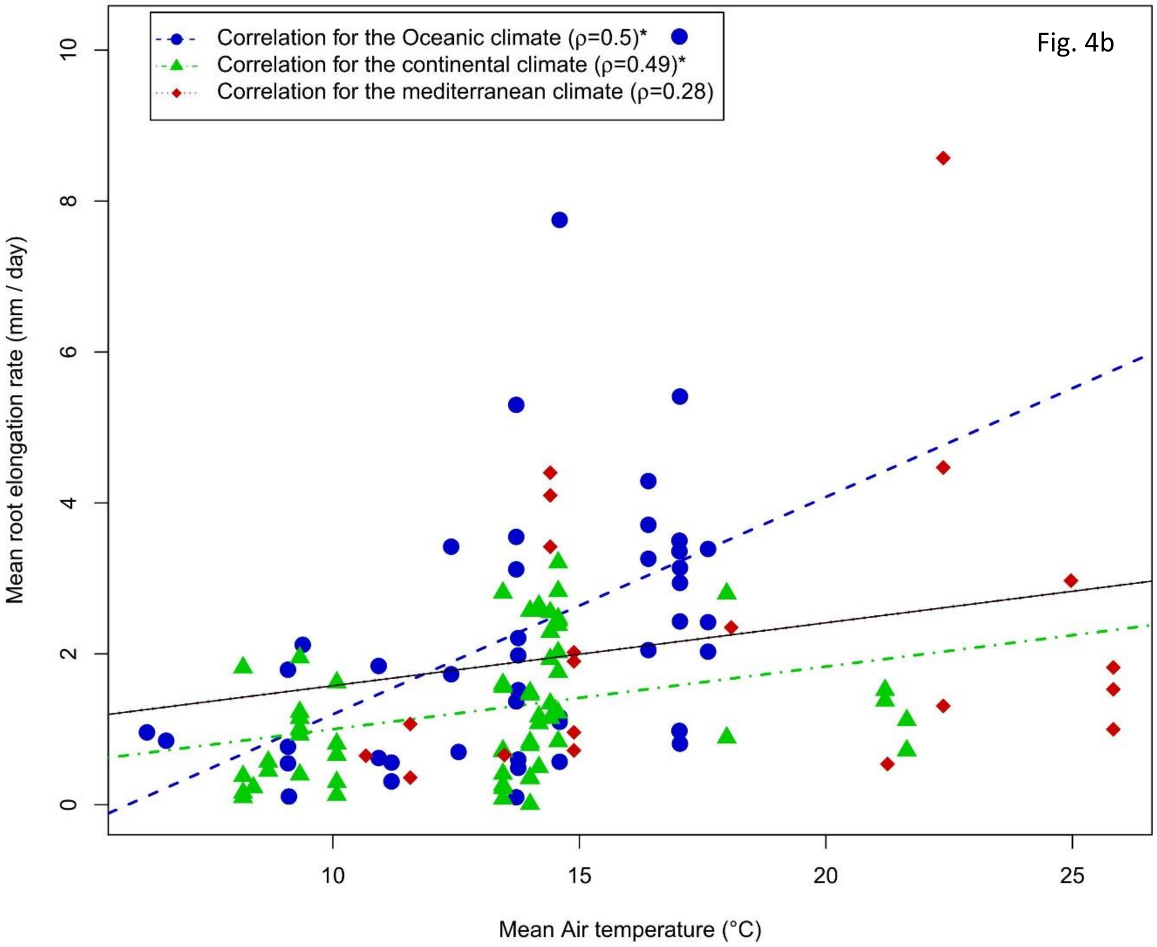


Fig 3: Comparison of roots (RER, grey area) and trunk (radial growth, green area) growth peaks in Mediterranean and Continental sites over the observation period from November 2014 to May 2016. Starting date for RER measurements in Mediterranean site is shown with a vertical dotted line.


Correlation for the Oceanic climate (ρ=0.56)*
Correlation for the continental climate (ρ=0.48)*
Correlation for the mediterranean climate (ρ=0.12)
Fig. 4a
Mean root elongation rate (mm / day)
0
2
4
6
8
10
8
10
12
14
16
18
20
Mean soil temperature (°C)


Correlation for the Oceanic climate (ρ=0.5)*
Correlation for the continental climate (ρ=0.49)*
Correlation for the mediterranean climate (ρ=0.28)
Fig. 4b
Mean root elongation rate (mm / day)
0
2
4
6
8
10
Mean Air temperature (°C)
10
15
20
25

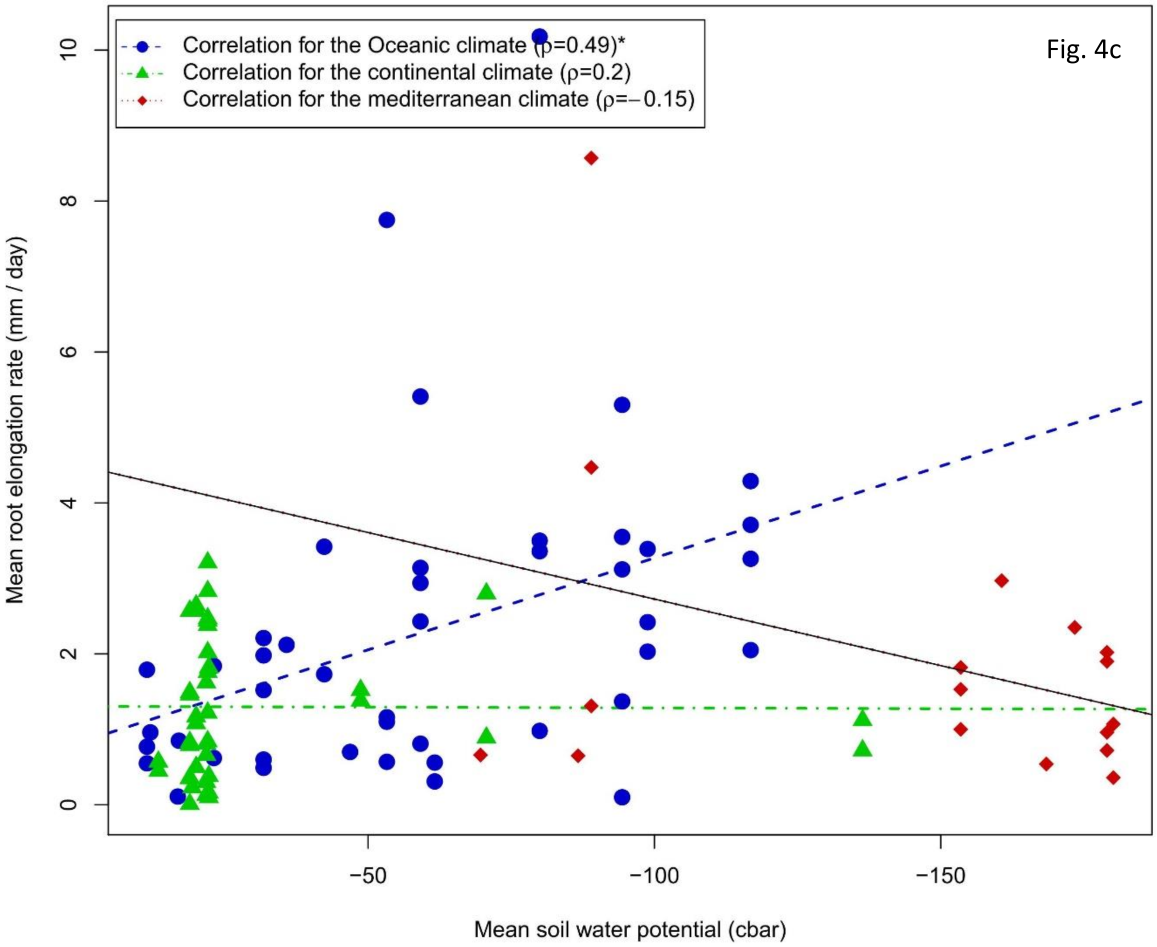
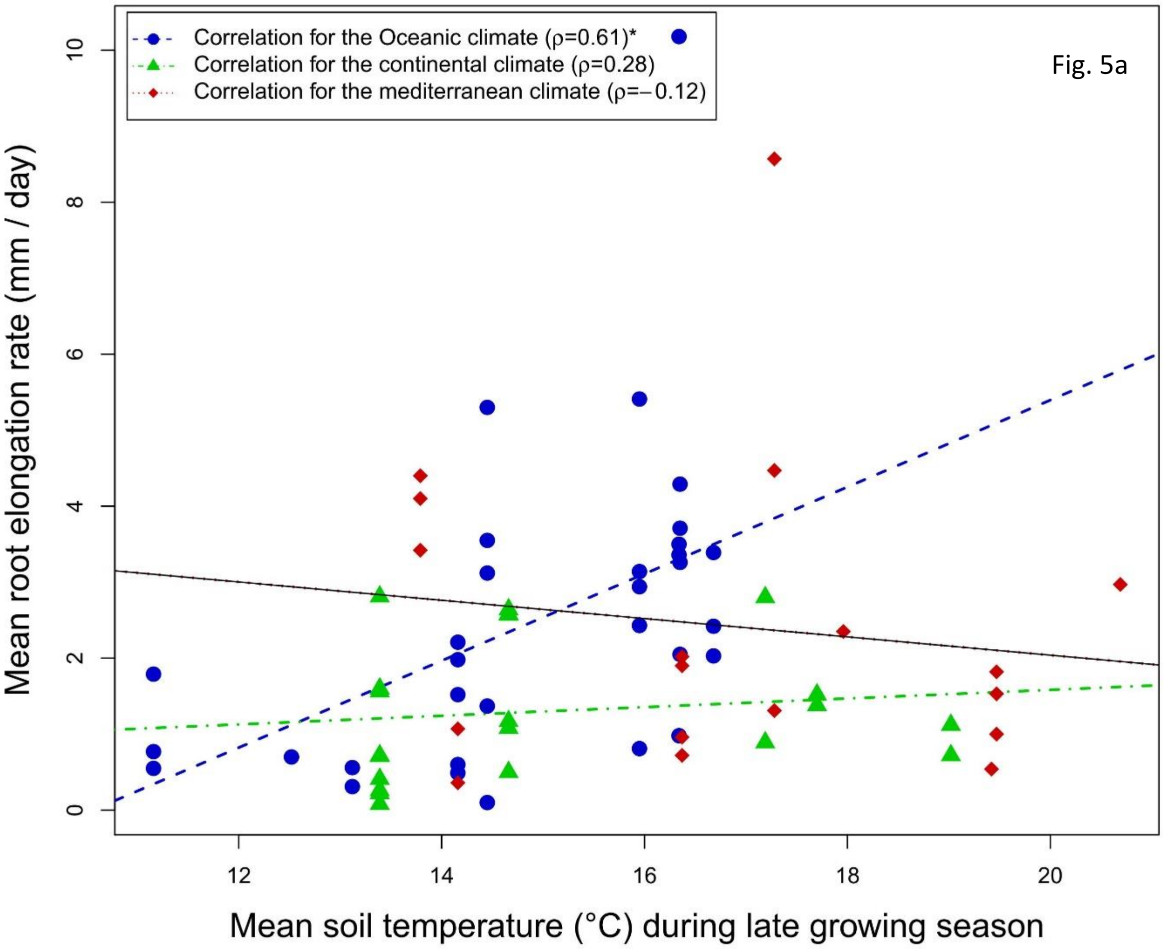
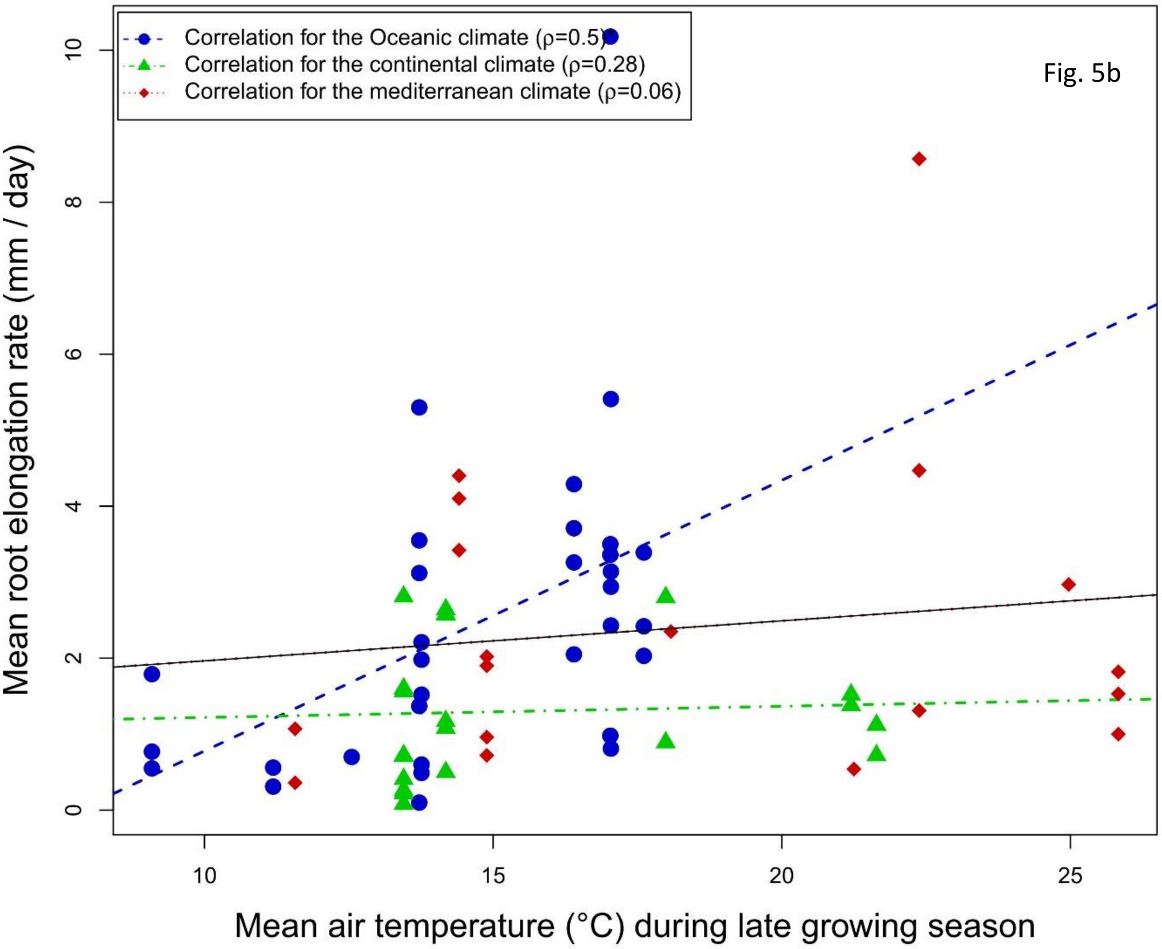


Fig. 4: Correlations (for all periods combined together) between mean root elongation rate (RER) and (a) mean soil temperature, (b) mean air temperature and (c) mean soil water potential at the oceanic (green triangles and dotdash line), continental (blue circles and dashed line) and Mediterranean (red diamonds and dotted line) sites.


Correlation for the Oceanic climate (ρ=0.61)*
Correlation for the continental climate (ρ=0.28)
Correlation for the mediterranean climate (ρ=−0.12)
Fig. 5a
Mean root elongation rate (mm / day)
0
2
4
6
8
10
12
14
16
18
20
Mean soil temperature (°C) during late growing season


Correlation for the Oceanic climate (ρ=0.5)
Correlation for the continental climate (ρ=0.28)
Correlation for the mediterranean climate (ρ=0.06)
Fig. 5b
Mean root elongation rate (mm / day)
0
2
4
6
8
10
10
15
20
25
Mean air temperature (°C) during late growing season

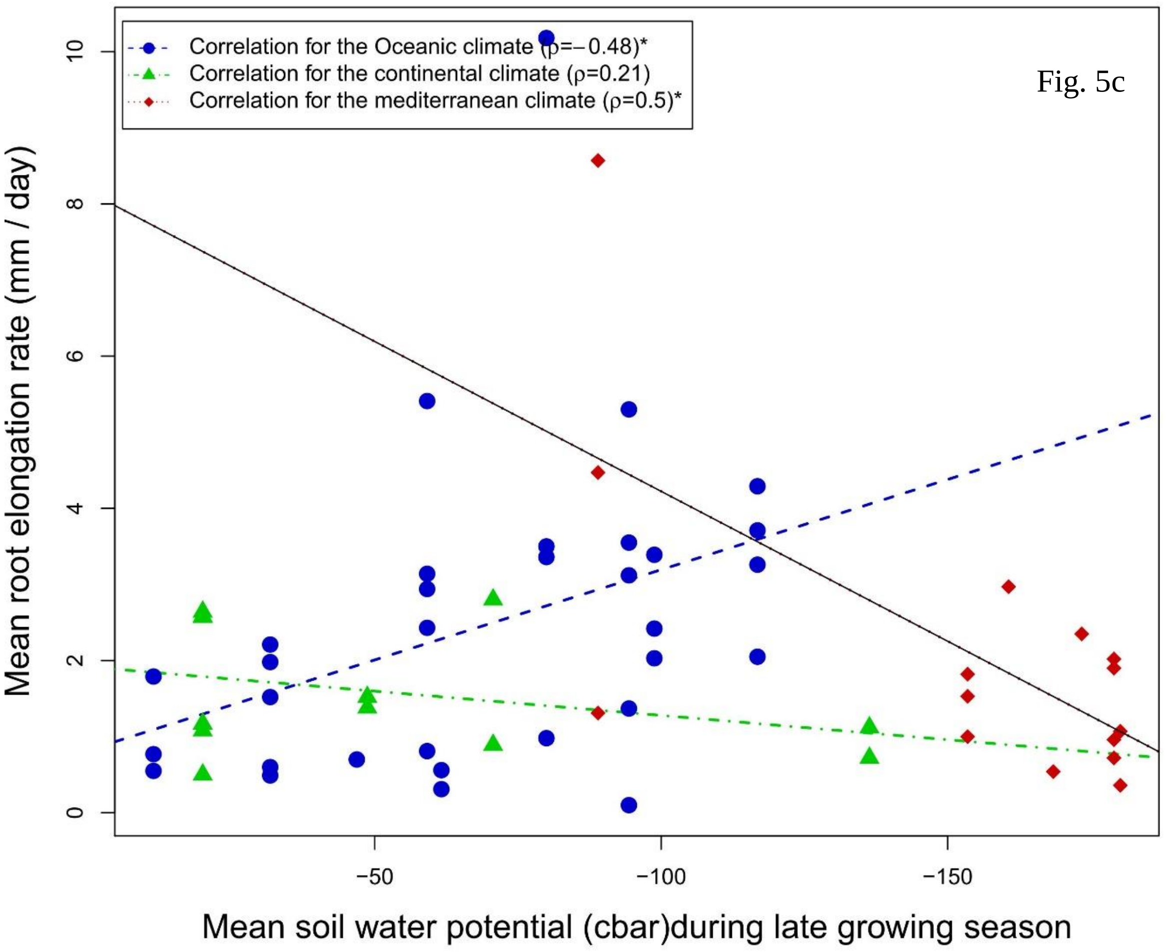


Fig. 5: Correlations (for late growing season only) between mean root elongation rate (RER) and (a) mean soil temperature, (b) mean air temperature and (c) mean soil water potential at the oceanic (green triangles and dotdash line), continental (blue circles and dashed line) and Mediterranean (red diamonds and dotted line) sites. Vertical bars represent standard deviations (not shown when smaller than the symbol size).

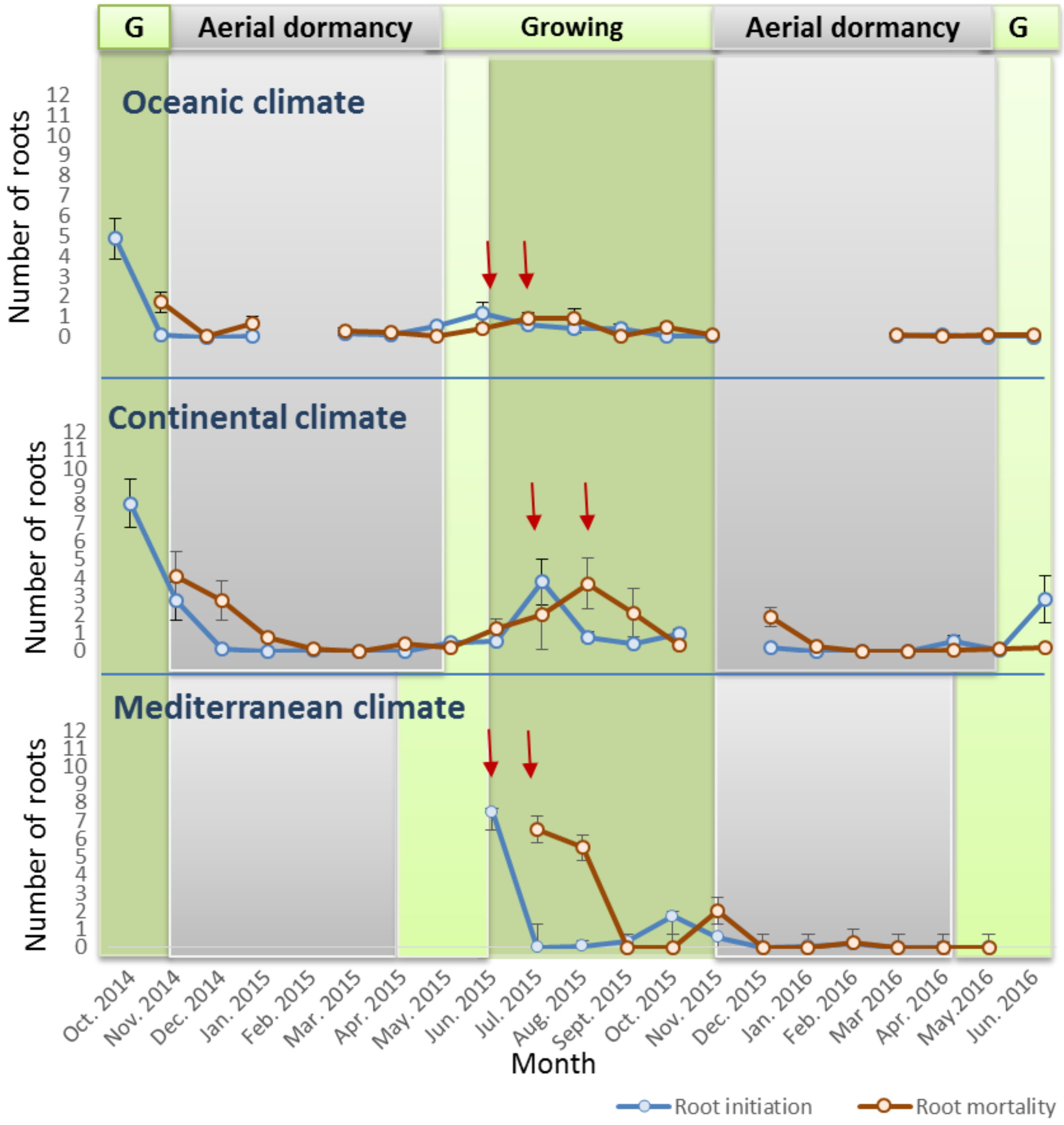


Fig. 6: Mean root initiation quantity (mRIQ, blue line) and mean root mortality quantity (mRMQ, red line) between time t and t-1, per 0.25 m^{-2} rhizotron (red line) at a) oceanic, b) continental and c) Mediterranean field sites over the observation period from September 2014 to June 2016. Different background colors correspond to different phenological periods: “LGS” is late growing season (green) “EGS” is early growing season (light green), “DS” is dormant season (gray). Missing data/ flood damage is shown with a dotted line over the dashed curves

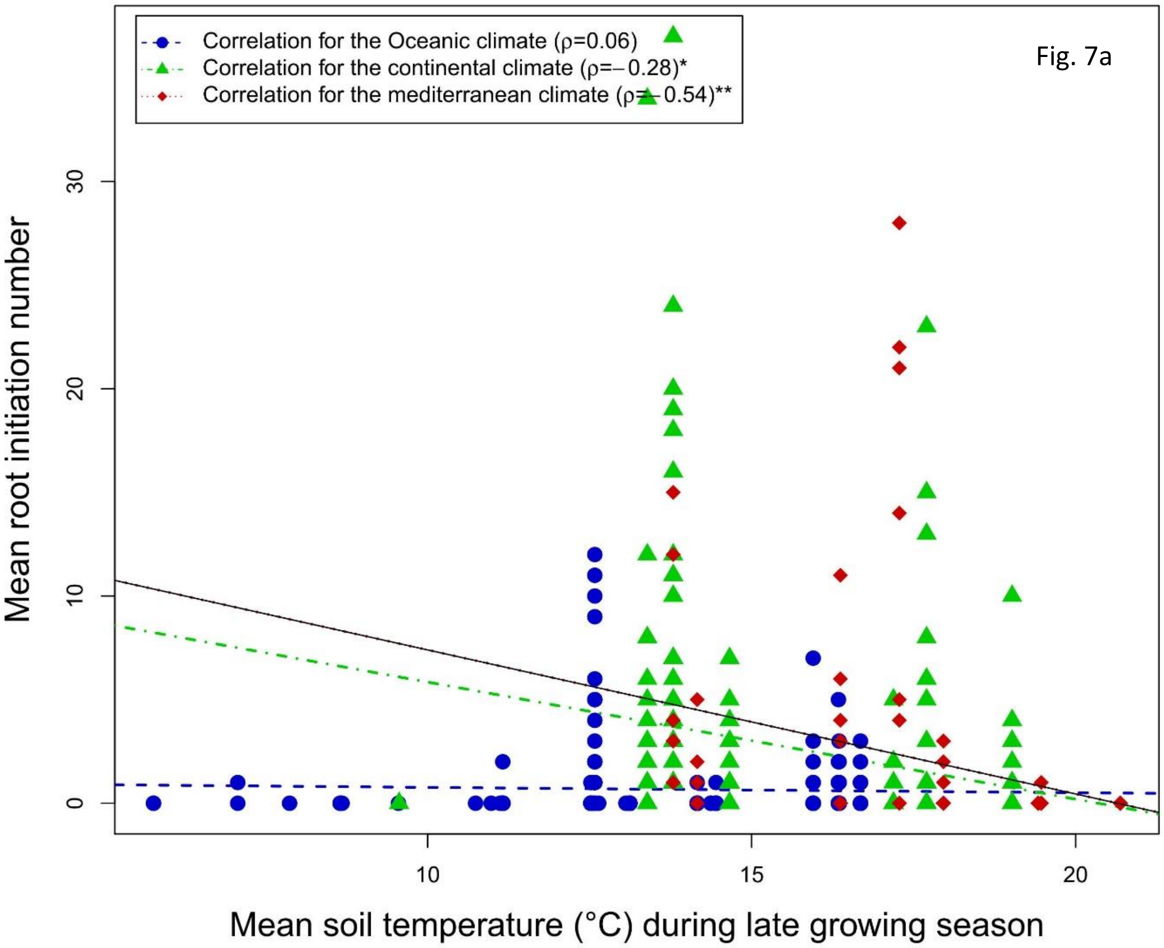
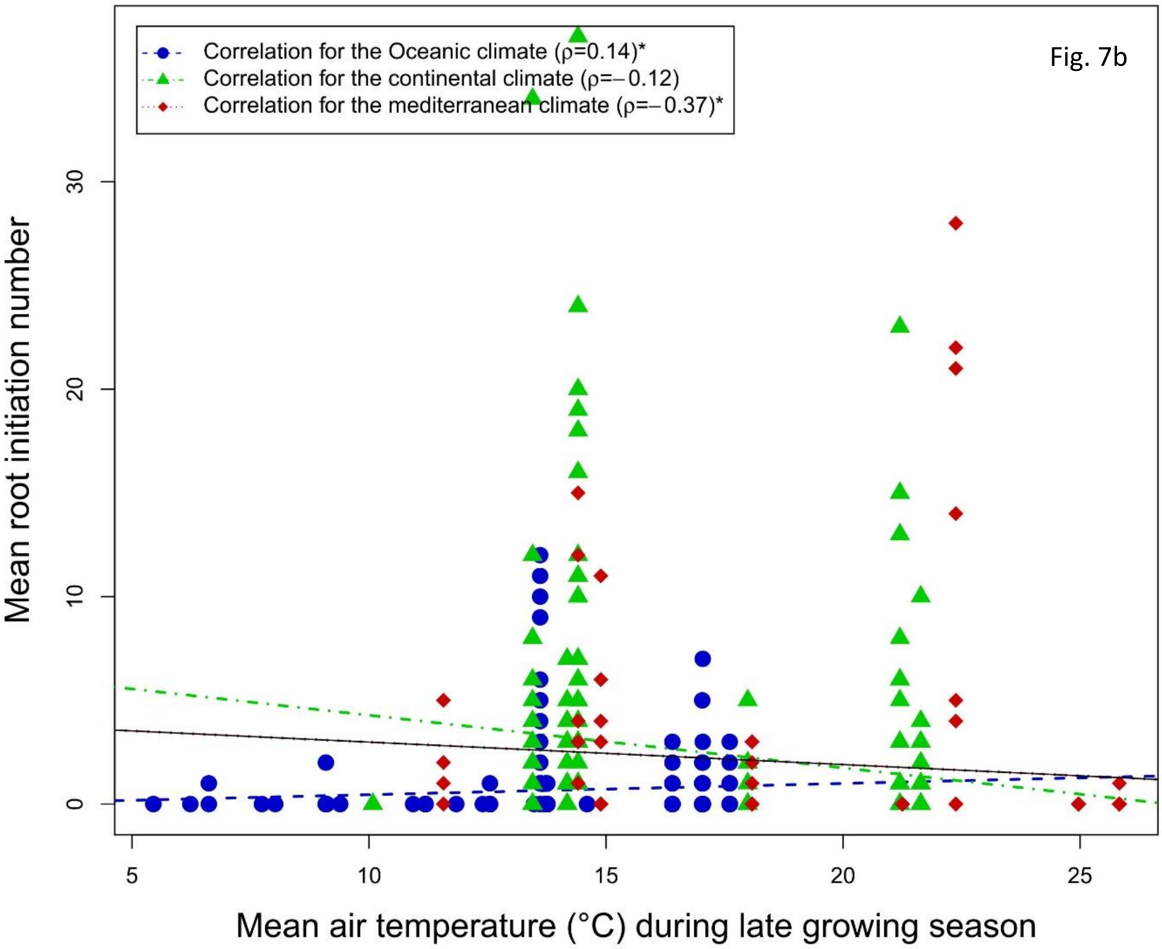


Fig. 7a

Correlation for the Oceanic climate (ρ=0.06)
Correlation for the continental climate (ρ=−0.28)*
Correlation for the mediterranean climate (ρ=−0.54)**
Mean root initiation number
0
10
20
30
10
15
20
Mean soil temperature (°C) during late growing season


Correlation for the Oceanic climate (ρ=0.14)*
Correlation for the continental climate (ρ=−0.12)
Correlation for the mediterranean climate (ρ=−0.37)*
Fig. 7b
Mean root initiation number
0
10
20
30
5
10
15
20
25
Mean air temperature (°C) during late growing season

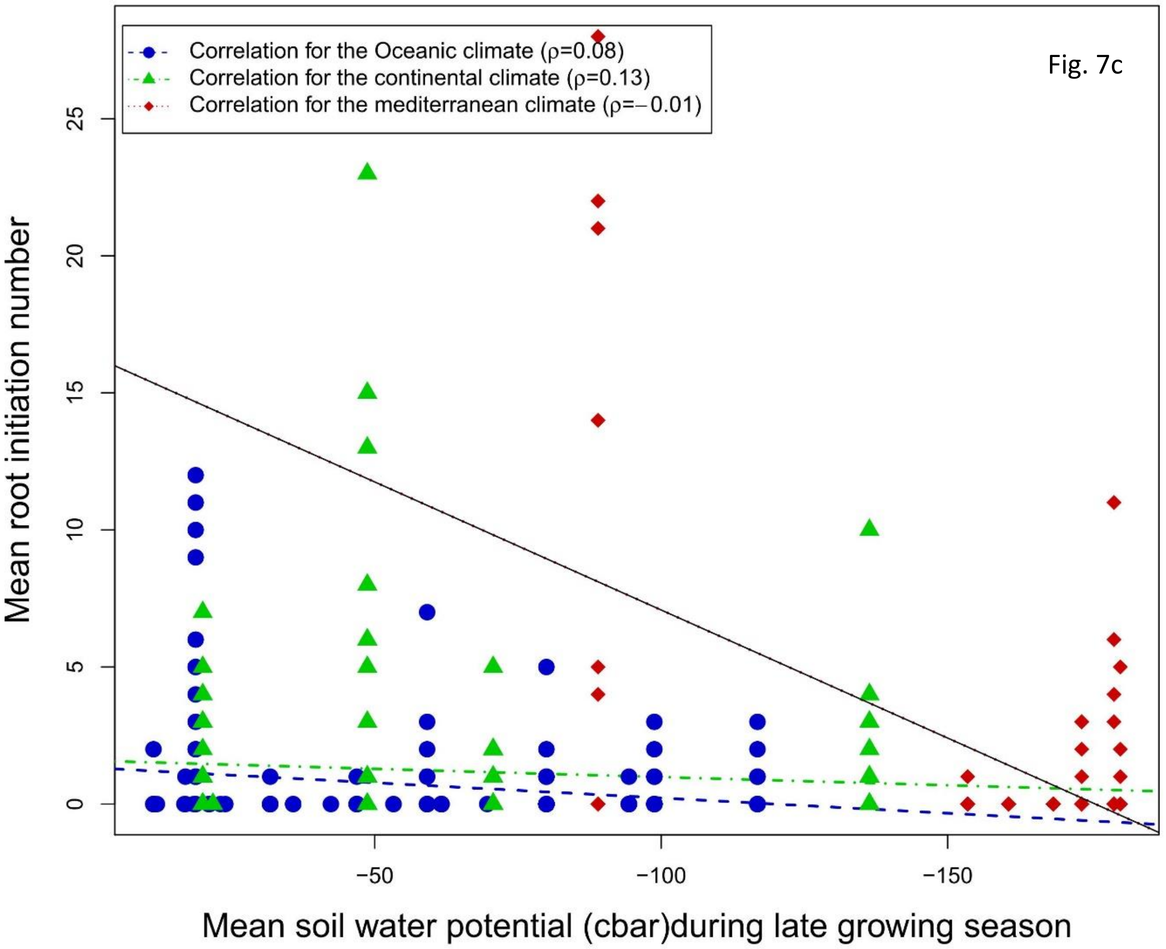
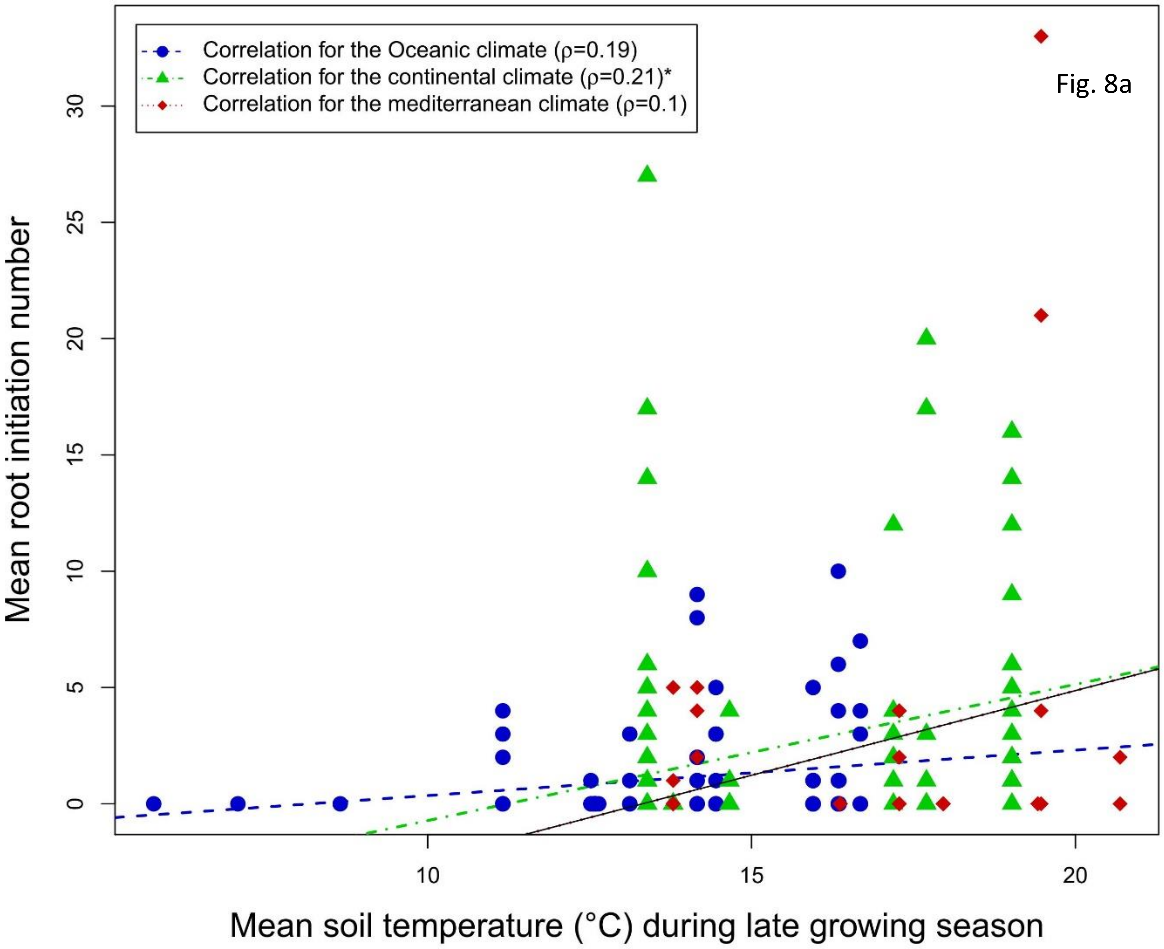


Fig.7: Correlations (for late growing season only) between mean root initiation (RIQ) and (a) mean soil temperature, (b) mean air temperature and (c) mean soil water potential at the oceanic (green triangles and dotdash line), continental (blue circles and dashed line) and Mediterranean (red diamonds and dotted line) sites.


Correlation for the Oceanic climate (ρ=0.19)
Correlation for the continental climate (ρ=0.21)*
Correlation for the mediterranean climate (ρ=0.1)
Fig. 8a
Mean root initiation number
0
5
10
15
20
25
30
10
15
20
Mean soil temperature (°C) during late growing season

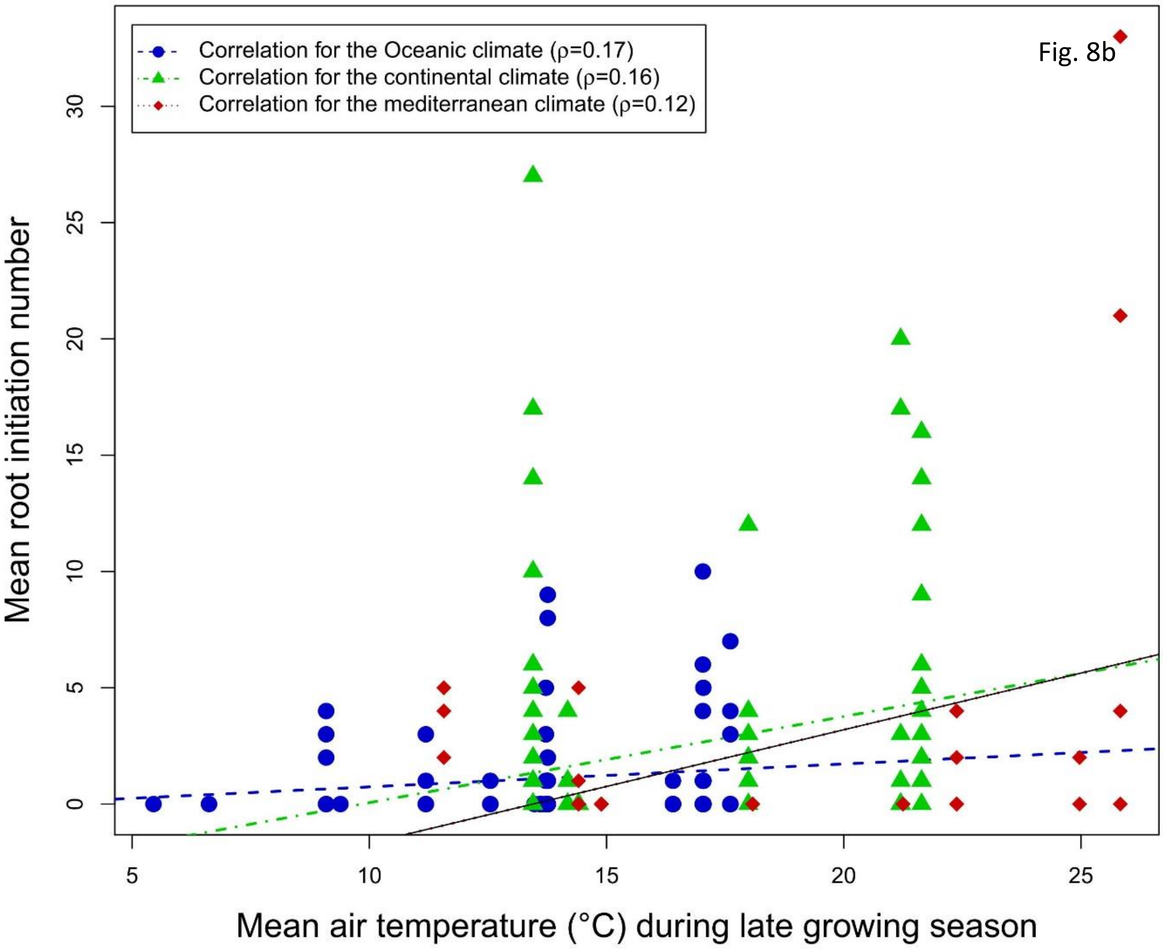


Fig. 8: Correlations (for late growing season only) between mean root mortality (RMQ) and (a) mean soil temperature, (b) mean soil water potential at the oceanic (green triangles and dotdash line), continental (blue circles and dashed line) and Mediterranean (red diamonds and dotted line) sites.

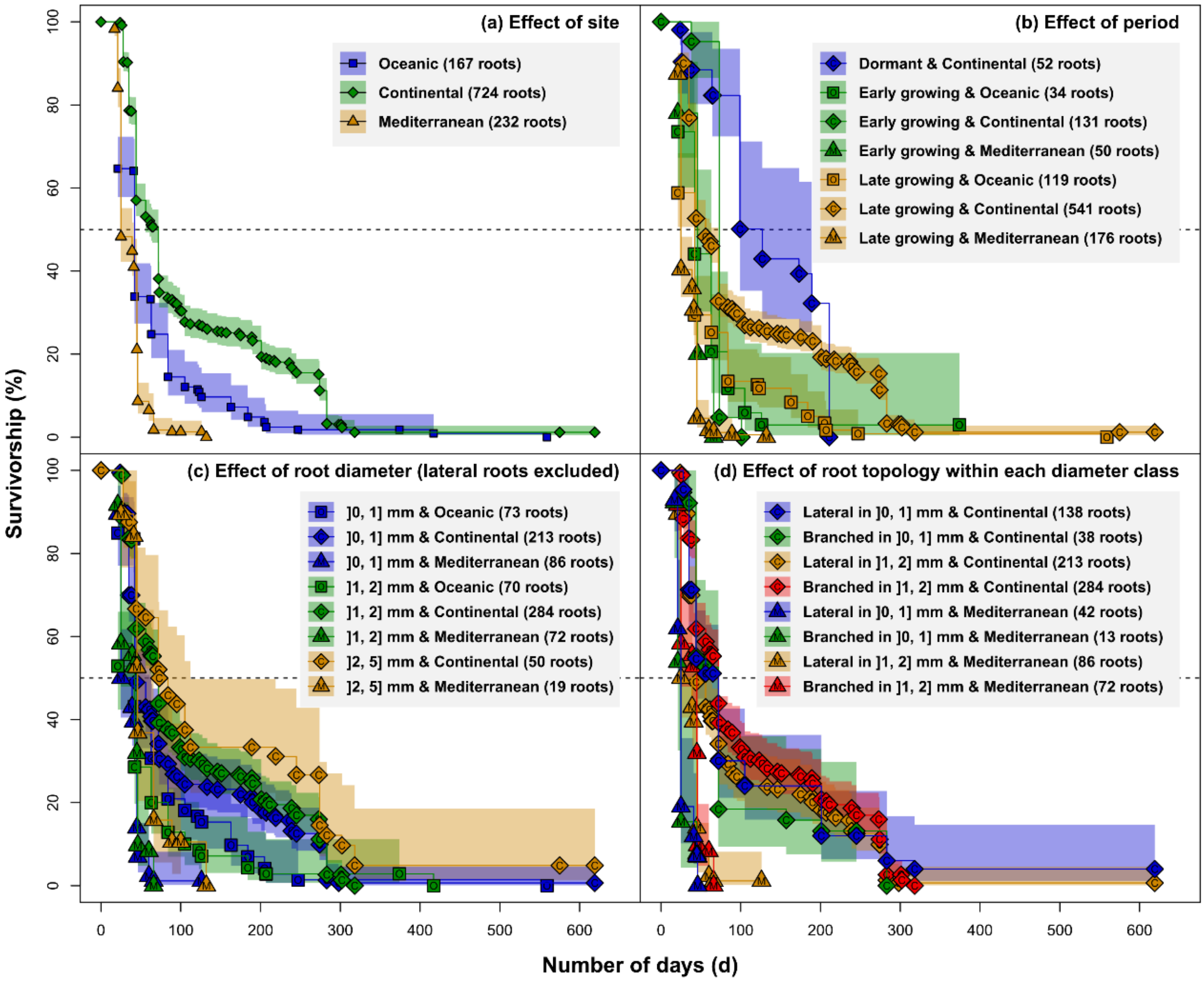
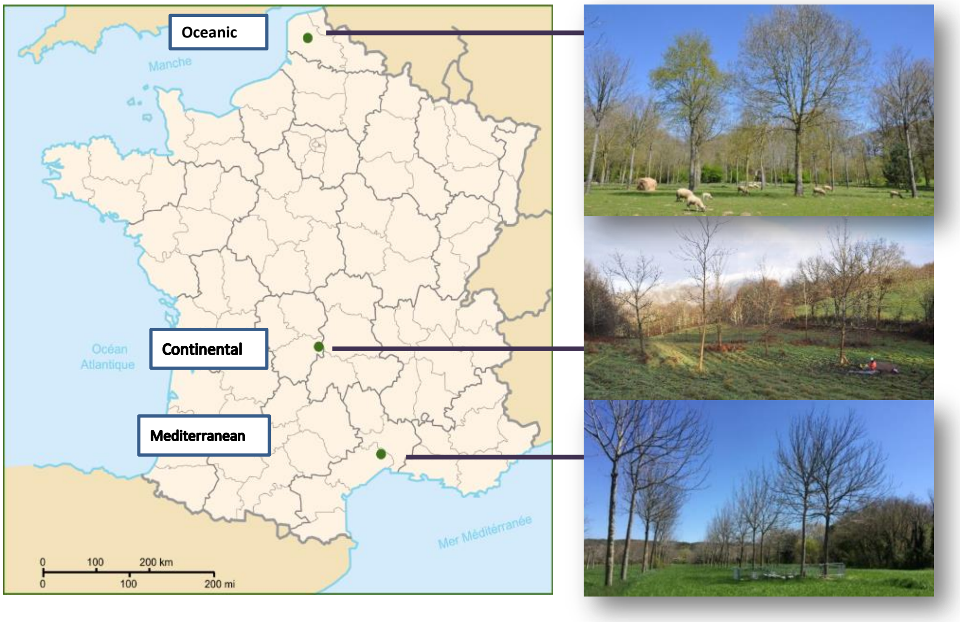


Fig.9. Cox's hazard regression relationships for estimating root survivorships in relation to (a) climate (oceanic - squares, continental - diamonds and Mediterranean - triangles), (b) phenological periods (early growing season, late growing season and dormant season), (c) root diameter classes (0-1) mm , (1-2) mm and (2-5) mm and (d) root topological order (first and second order roots) over the observation period from October 2014 to June 2016. Different shades represent the interval confidence of each curve corresponding to the color of that curve.

8. Appendix

Appendix 1: Aerial view of the three sites taken by google earth

Appendix 2: The three agroforests system along a latitudinal gradient in France



Appendix 3: Thermometer i button (DS1921G Thermochron ibuttons)

Appendix 4: Watermark monitor to measure soil water potential

WATERMARK Monitor

Appendix 5 : Rhizotrons installed in different sites

Appendix 6 : The grand pit at Mediterranean site

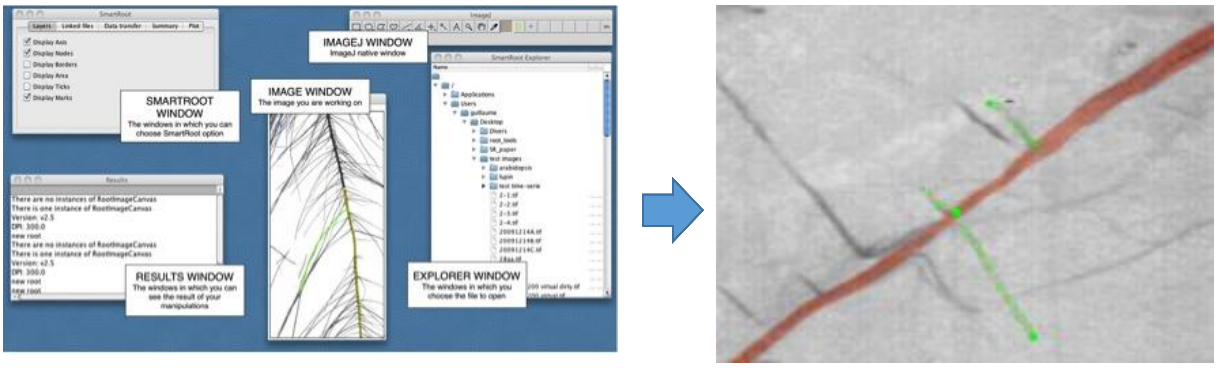
Appendix 7: Epson Perfection (V370) Flatbed scanner to measure root growth through rhizotrons

Appendix 8: Time-lapse camera to take photos of root system through rhizotron

Appendix 9: Flood damage at both (A) Mediterranean and (B) oceanic sites

Appendix 10: SmartRoot software for analyzing images of roots

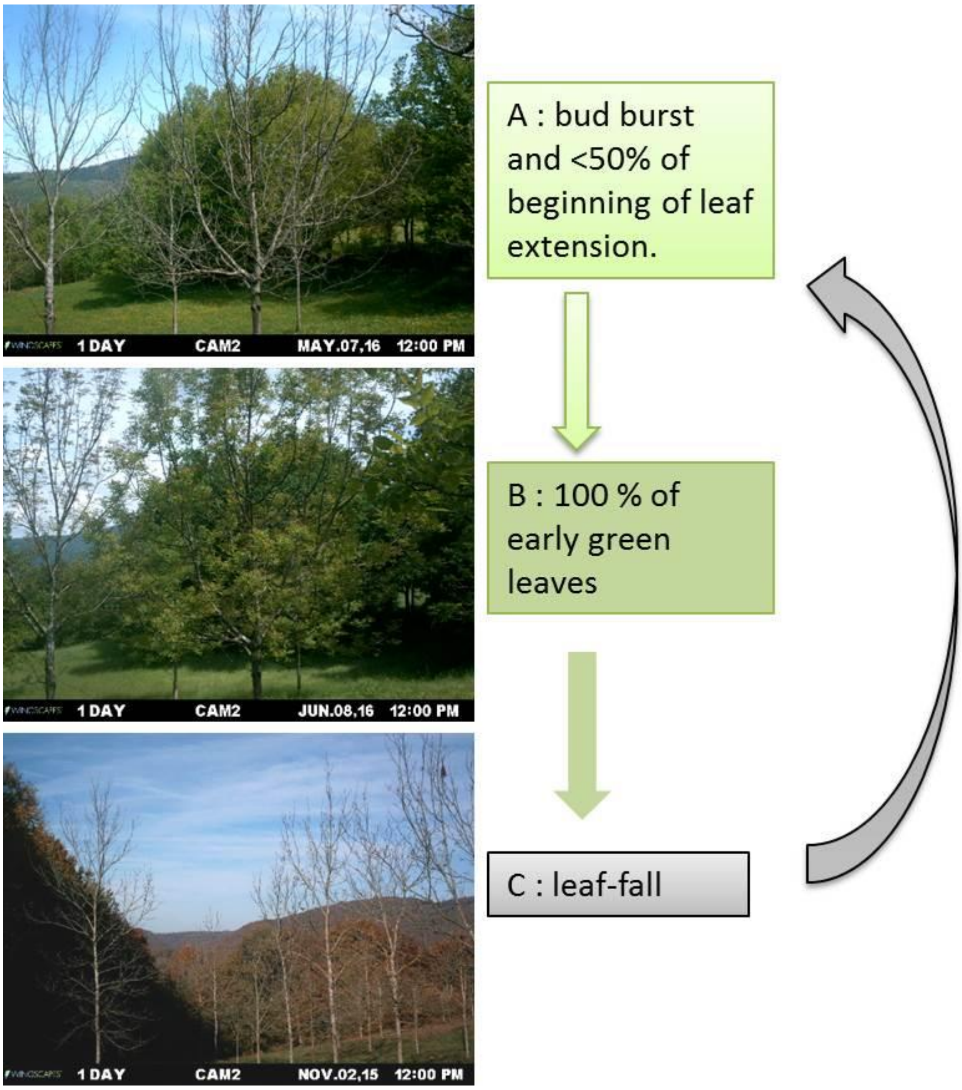
SmartRoot software



☑ Display Axis
☑ Display Nodes
☐ Display Borders
☐ Display Area
☐ Display Ticks
☑ Display Marks

```
There are no instances of RootImageCanvas
There is one instance of RootImageCanvas
Version: v2.5
DPI: 300.0
new root
There are no instances of RootImageCanvas
There is one instance of RootImageCanvas
Version: v2.5
DPI: 300.0
new root
new root
```

Appendix 11: the three phenological periods throughout the year



A : bud burst and <50% of beginning of leaf extension.

B : 100 % of early green leaves

C : leaf-fall

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Chapter IV: Shoot and root phenological relationships in hybrid walnut growing in a Mediterranean alley cropping system

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Abstract

Plant phenology is crucial to assess the impact of climate change on plant productivity and species' distribution. Despite the key role of fine roots in carbon and nutrient cycling in ecosystems, root phenology has been less well characterized than shoot phenology due to the methodological problems in measuring root systems. These problems have also made it challenging when observing fine roots at depths >1.0 m, as well as relating belowground phenology to the better established patterns of above ground phenology. We examined the influence of climatic factors on fine root elongation rate (RER), to a depth of 5 m, and assessed relationships with shoot phenology of walnut trees (*Juglans nigra* x *Juglans regia* L.) in a Mediterranean agroforestry system. Rhizotrons and minirhizotrons were installed for 21 months to monitor root elongation rate. Dendrometers were used to measure stem and root radial growth during the same period. Fine RER was not synchronous with leaf phenology at any soil depth. However, RER in the two upper soil layers (0.0-0.85 m and 0.85-1.7 m) was synchronous with both stem and root radial growth during late growing season only, which were positively correlated with mean soil and air temperature and solar irradiance. Mean RER was not correlated to either soil and air temperature or to soil water content at any soil depth during the late growing season. However, mean RER of shallow roots (0.0-0.85 m) was significantly and positively correlated with mean solar irradiance. The timing of the maximal peak rate of elongation differed between soil layers and decreased with increasing soil depth. Maximal RER was found during the late growing season for the three upper soil layers whilst roots at the deepest soil layer (4.0-4.7 m) peaked during the dormant season (December). We conclude that shoot and root growth are under different controls and that drivers of shallow and deep roots are not the same.

Key words:

phenology, *Juglans* L., root elongation rate, deep roots, stem growth, structural root growth

1. Introduction

In the current context of global climate change, predicting the response of ecosystems and vegetation to climate variations has become a major challenge for research (Casassa et al 2007). Plant phenology is crucial to assess the impact of climate change on plant productivity and species distribution (Chuine 2010, Chuine & Cour 1999). Observations of tree developmental stages are considered to be one of the most reliable indicators of climate change (Slaney et al 2007) (Donnelly et al 2006). Most studies on plant phenology have focused on shoot phenology, which is more easily accessible and plays an important role in determining species' responses to climate change (McCormack et al 2014). However, much less is known about root phenology due to methodological problems in measuring unseen roots without disturbing the system of study (Norby & Jackson 2000). This difficulty has also made it challenging to relate belowground phenology to better established patterns of above ground phenology (McCormack et al 2014). Fine roots play a key role in ecosystem functions and nutrient cycling and have a significant effect on soil carbon (C) sequestration, especially in deep soil layers (Cardinael et al 2015, Kell 2012).

Root phenology differs by depth in certain ecosystems and has different controls (Canham et al 2012, Germon et al 2016). However, most studies on fine root phenology in temperate ecosystems have been performed in superficial layers of soil (<1 m) (Hendrick & Pregitzer 1993). Knowledge about root phenology in deeper soil layers remains scanty. Moreover, stems and structural roots are the critical link between roots and leaves and serve transport, storage and support functions (Gartner 1995). Knowledge about their phenology is important in determining the amount of carbon allocation from shoots to roots. Although aboveground phenological events include bud burst, leaf expansion and leaf fall have attracted much attention (McCormack et al 2015) (Sloan et al 2016). However, studies of drivers that control stem and structural root growth and phenology remain scarce.

Agroforestry systems associating trees with farming practices (Somarriba 1992) (Cardinael et al 2015) play an important role in climate change mitigation and adaptation (Ramachandran Nair et al 2009). The alley cropping system is one of the most established agroforestry practices in temperate regions (Cardinael et al 2015). The interaction between trees and crops in such systems protect the environment and offer a number of ecosystem services (Jose 2009) (Newaj et al 2016). Trees contribute to soil C enrichment through root turnover, increasing water infiltration and retention in the soil profile by increasing soil porosity and reducing runoffs (Newaj et al 2016) (Gyssels et al 2005). In addition, tree roots are able to expand over a large volume of soil for water and nutrient uptake and in doing so, increase soil aggregate stability, perform hydraulic redistribution and take up a higher volume of soil water and nutrients which are less accessible to shallow-roots crops (Udawatta et al 2002), and thus improve soil fertility (Buck et al 1998). Yet there are still major uncertainties about the mechanisms that control fine root phenology in these systems, especially in deep soil horizons.

Plant phenology can be controlled by both biotic and abiotic factors. Temperature is reported to be the most important abiotic factor controlling spring phenology in temperate climates (Wielgolaski 2003). Air temperature is considered to be the most important environmental factor controlling the timing of aboveground growth (Menzel 2003) (Radville et al 2016) (Wielgolaski 1999). Factors controlling below ground phenology especially in deep soil horizons are still unclear. For example, (Germon et al 2016), studying hybrid walnut (*Juglans nigra x Juglans regia* L.) in a Mediterranean climate found that root elongation rate (RER) was under soil temperature control. Whereas, Misson et al.(Misson et al 2006) studying *Pinus ponderosa* in a Mediterranean climate also found that root growth was controlled by soil water content. Many other studies failed to find a correlation with abiotic factors and have suggested that endogenous factors and allocation of stored carbohydrate are the drivers of root growth in Mediterranean climate (Abramoff & Finzi 2015).

Shoot and root growth and phenology may be closely related because shoots are dependent on roots for soil resources and roots are dependent on shoots for photosynthates (Steinaker et al 2010). Thus, knowledge about the timing of both shoot and root phenologies may improve our understanding of whole plant functioning (Du & Fang 2014). Some studies showed that shoot and root production are synchronous (Germon et al 2016) (Misson et al 2006). While other studies demonstrated that shoot and root production are asynchronous. Shoot production may precede root production by several weeks or months i.e. in Mediterranean, arctic, boreal and temperate biomes (Abramoff & Finzi 2015). In contrast, root production may precede shoot production in other biomes (e.g., subtropical biome) (Abramoff & Finzi 2015). The possible reason of the asynchrony between root and shoot phenology may refer to that above and belowground organs are either under different controls or that competing for carbon-use (Radville et al 2016). Understanding environmental factors and source–sink relationships controlling both root and shoot growth is fundamental to understanding how plants may adapt to a changing climate. Hence, synchronous measurements of different plant organs (leaf, stem structural roots and fine roots) are essential to a better understanding of the seasonal C allocation within a plant and therefore whole plant responses to climate change (Sloan et al 2016) (Steinaker & Wilson 2008).

The RER is one of the most important root phenology indicators, is a consequence of both cell divisions on the meristem and cell elongation in the elongation zone (Steinaker & Wilson 2008). The important role of this trait is its plasticity in the soil matrix with regard to water and nutrient uptake, thus maintaining whole plant function (Mao et al 2013b). RER may vary between classes of diameter or branch order (Germon et al 2016) (Mao et al 2013a). These variations in RER can affect tree's C allocation by influencing the rhythm of root respiration (Mao et al 2013a).

In this study, we examined fine RER of growing roots in different soil depth (down to 4.7 m) of walnut trees (*Juglans nigra x juglans regia* L.) growing in an alley cropped Mediterranean agroforest system, for 21 months. We focused on relating fine root phenology to leaf, stem and structural root phenology. We asked whether shoot phenology reflects or differs from fine root phenology in different depths. Root phenology measurements were performed using rhizotrons and minrhizotrons installed in a large pit (to a depth of 4m). We hypothesized that (i) shoot and root production is asynchronous and under different controls regardless of soil depth, (ii) the drivers of fine root elongation are not the same between soil depths.

2. Materials and methods

2.1.Study site

The agroforest was located at the Restinclières experimental site, 15 km north of Montpellier, Department Hérault, France (43°43'N, 4°01'E, elevation 54 m a.s.l.) (Appendix 1). The climate is sub-humid Mediterranean with a mean annual temperature of 15.4 °C and a mean annual rainfall of 873 mm. The study plot comprised hybrid walnut trees (*Juglans nigra* × *regia* L. cv. NG23) (at 13 × 4 m tree spacing) intercropped with durum wheat (*Triticum turgidum* L. subsp. *durum (Desf.*) Husn). However, rapeseed (*Brassica napus* L.) was also cultivated in 1998, 2001 and 2006, and pea (*Pisum sativum* L.) in 2010 and 2016. All walnut trees were planted in 1995 in lines oriented east west, and the current density is of 110 trees ha^{-1}. The annual crop was fertilized with approximately 150 kg N ha^{-1} yr^{-1} (Cardinael et al 2015). The soil is a silty clay deep alluvial soil (25% clay and 60% silt) (depth > 4m) and slope angle is <1° within the site (Mulia & Dupraz 2006). The average pH is 8.0 (Dupraz et al 1999). The site is near the Lez river watershed and the depth from the soil surface to the water table usually oscillates between 5 m in winter and 7 m in the summer (Cardinael et al 2015).

More details about the experimental site are available in (Dupraz et al 1999) (Mulia & Dupraz 2006) (Cardinael et al 2015). Mean DBH of all walnut trees at the site was 0.24 ± 0.13 m and mean height was 11.09 ± 2.50 m.

To measure walnut root elongation, we installed rhizotrons and minirhizotrons, also called root windows. Rhizotrons and minrhizotrons comprised transparent polyvinylchloride (PVC) sheets and tubes, respectively, placed against the soil profile, through which root growth dynamics can be observed (Reich et al 1980) (Misra 1999) (Mao et al 2013a) (Germon et al 2016). One pit (5 m length x 1.5 m width x 4 m depth) was dug in March 2012 between two walnut trees on the same tree row (Appendix 2) (Cardinael et al 2015). The pit was reinforced with wooden beams to avoid collapse of the pit walls. In November 2011, six tubes (105cm length, 7.6 cm diameter) were installed in the field between two trees on different lines of trees. In June 2012, 16 other tubes (105cm length, 7.6 cm diameter) were installed at depths of 0.0, 1.0, 2.5, 4.0 m. Moreover, in June 2014, two rhizotrons (100 cm long x 80 cm wide x 0.5 cm thick) were installed as well as eight smaller windows (50 cm long x 30 cm wide x 0.5 cm thick) at depths of 20, 110, 200 and 280 cm.

Where the rhizotrons were to be placed on the soil wall, we gently removed the soil to make a flat surface and cut all roots on the profile with secateurs. The soil removed during the digging of the trenches was kept aside, and then sieved through a 5 mm sieve and air-dried for several hours. The sieved and air-dried soil was then poured into the space between the window and the soil profile and slowly compacted using a wooden plank. Rhizotrons were placed vertically against the soil profile at an angle of 15°. While tubes were inserted to soil profile of the pit at an angle of 45° using a drill permitting to measure a surface of 0.7m of soil profile. These angles will permit the roots to grow downwards due to positive geotropism (Mao et al 2013a) (Huck & Taylor 1982). Each rhizotron was covered with foil backed felt insulation and black plastic sheeting to protect roots from light and temperature variations. As well as, each tube was closed with black caps to prevent light entering the tubes. The pit was then covered with a metallic roof to avoid damage from passing animals and to prevent direct rainfall and sunlight on both rhizotrons and minirhizotrons (Appendix 3). In September 2014, the trench was flooded due to exceptionally strong rainfall (Appendix 4) therefore all rhizotrons only were reinstalled in March 2015. No data were recorded from September 2014 to March 2015, and data were recorded but not used in statistical analysis from March to June 2015 (the three months safety period after rhizotron installation (Strand et al 2008). Monthly measurements of root elongation rate were carried out immediately after roots were observed in each rhizotron (and after the first three months had passed) until June 2015.

2.2.Measurements of root growth

To measure fine root growth dynamics, we either scanned rhizotrons or minrhizotrons before then analyzing images (Mohamed et al 2016, submitted). A scan of each rhizotron (between two and four images per window depending on the size of the window) was taken at monthly intervals over 12 months using an Epson Perfection V370 flatbed scanner with a high optical resolution of 4800 dpi (Appendix 5a). Similarly, a scan of each minirhizotron (five images of 21.59 × 19.56 cm per tube) was taken at monthly interval over 22 months using a circular scanner (CI-600 Root Growth Monitoring System, CID, USA) (Graefe et al 2008) that runs around through the tube (Appendix 5b).

2.3.Climatic data measurements

Global precipitation and air temperature data for the site was obtained from Station Restinclières, 43.702 N et 3,86 E, elevation 100 m a.s.l.). In April 2013, eight volumetric soil moisture sensors (Campbell CS 616, France) and eight temperature sensors (Campbell 107, France) were installed in the pit near the minirhizotrons tubes at four depths (0.5 m, 1.2 m, 3 m and 4 m) (Cardinael et al 2015). Soil temperature and volumetric moisture were recorded every hour using a Campbell dataloger (CR1000) (see Geromn et al, 2016, Cardinael et al, 2015 for more details).

2.4.Image analysis

Once images of root growth had been acquired, we conducted image analyses using the semi-automated SmartRoot software (Lobet et al 2011) (Appendix 6). SmartRoot is an operating system independent freeware based on ImageJ and using cross-platform standards (RSML, SQL, and Java) for communication with data analysis softwares (Lobet et al 2011) (Mathieu et al 2015). Before analyzing roots, it was necessary to stitch images together (e.g. with Adobe Photoshop CS3 software) if several have been taken for the same rhizotron or minirhizotrons (because the windows surface area was greater than the field of the scanners). We transformed all images to 8 bit gray scale and then inverted them using ImageJ software so that roots were darker than the background of the image. The length and diameter of each root produced during one interval time (i.e. one month) were calculated for each window. Before analyzing a new sequence of images, SmartRoot provides the user with an icon to import the previous data file on this new image, which helps the estimation of the evolution of root length. This preceding image also helps determine whether the root is live (usually cream in color) or dead (turning black) (Huck & Taylor 1982) (Mao et al 2013b).

2.5.Aboveground phenology and stem growth

We assessed the timing of shoot production through changes in the phenological phases of leaves throughout the year. We divided the year into three phenological periods, early growing season (from budburst to 100% leafing out of early green leaves), late growing season (100% leafing out to leaf-fall) and dormancy (leaf-fall to budburst). Shoot phenological periods were recorded visually using binoculars.

We also measured the radial growth of tree trunks by installing dendrometers (Increment Sensor DB20) on eight trees. We also installed dendrometers on five excavated structural roots of two trees. The dendrometers were girth bands, comprising a steel band, spring and nonius scale. Bands were placed on the stem; at a height of 1.3 m. Measured values are read at the nonius scale with 0.1 mm precision (Appendix 7). Readings were made monthly. Tree height was recorded annually after leaf shedding using a hypsometer Vertex (Quebec, Canada).

2.6. *Root growth dynamics*

We used the following methods to estimate root elongation rate:

(i). Individual root growth was evaluated by calculating the difference between the root length at initiation ($t_{-1)}$ and at the day on which the measurement was made (t) To determine the daily root elongation rate (RER), the mean of all individual root lengths produced between time t and t_{-1} was divided by the duration of the corresponding period.

$$RER_{t-1,\,t} = \frac{len._{t} - len._{t-1}}{Pt\text{-}t_{-1}}$$

Where, $RER_{t-1,t}$ is the daily RER for all roots undergoing growth (zero values were excluded); $len._{t-1}$ and $len._{t}$ are the lengths of the root n at inventory time t_{-1} and t, respectively; $p_{t-1,t}$ is the period between inventory time t_{-1} and t.

2.7. *Statistical analysis*

Mean root elongation rate (RER was calculated using R software.Version 2.15.3 (R Development Core Team 2013). RER for growing roots only was performed in the statistical test. A Shapiro-Wilk test was performed before each test to ensure if the investigated indicator followed a normal distribution and homogeneity of variances was checked. For data not normally distributed, analyses were performed by a Kruskal-Wallis Test. Effects of season, soil depth, diameter classes were tested on root elongation rate. A post-hoc analysis was performed when Kruskal-Wallis result was significant using Nemenyi test of Kruskal Wallis at $p<0.05$ to determine which levels of the independent variable differ from each other level. Box plots were used to describe root growth between soil depth and class of diameter, data are mean +/- standard error. Spearman's rank correlations (Spearman) were performed to determine the relationship between the climatic variables (mean soil and air temperatures and mean volumetric water content Θ) and mean RER. The total surface size and total number of growing roots observed along the study period differ between soil depths (respectively 74 roots in 1,58m² for depth (0-0.85); 49 roots in 1,54 m² for depth (0.85-2.00); 24 roots in 2.12m² for depth (2.00-3.30 m) and 22 roots in 0.84 m² for depth (4.00-4.70). All analyses were performed using R software, Version 2.15.3 (R Development Core Team 2013) at a significance level of <0.05.

3. Results

3.1 Meteorological and soil hydrological data

Mean soil temperatures from the upper to lower depths of soil over the two-year period were 14°C, 13.7°C, 13.5°C, 13.4 °C. Mean soil temperature was significantly higher at both 0.0-0.85 m and 0.85-1.7 m depths than at the two deeper soil horizons. There were no significant differences in soil temperature between the two upper soil horizons (0.0-0.85 m, 0.85-1.7 m), nor between the two deeper soil horizons (2.0-3.3 m, 4.0-4.7 m) during the aerial late growing season (Fig.1). Soil volumetric water content, θ, increased significantly with augmenting soil depth ($p<0.001$) with mean θ of 27.9%, 29.2%, 31.9%, 33.2 %, from the upper to lower layers. However, no significant differences were found in θ between 2.0-3.3 m and 4.0-4.7 m soil layers. Precipitation was highly variable between the two years, with 1264 mm in 2014 and only 613 mm in 2015. The sum of precipitations during the aerial late growing season in the period of the entire study was 1053.2 mm and decreased to 654.3 mm during the dormant season. Mean global solar irradiance was 0.18 kw/m^2 in 2015 and was 0.19 kw/m^2 during the aerial late growing season over the entire study period.

3.2 Leaf, stem and root phenology

Budburst occurred on 18 and 14 April in 2015 and 2016, respectively. 100 % of leaf unfolding occurred on 02 June 2015, and leaf shedding occurred on 18 and 12 November in 2014 and 2015 respectively. Radial stem growth preceded radial root growth by one month and both peaked in June or July before decreasing, and then increasing to a greater extent in September. Radial root growth started when trees had 100 % of green leaves unfolding, corresponding to the onset of the late growing season (02 June 2015). Both stem and root radial growths dropped sharply with leaf fall. Radial root growth followed a similar pattern to that of radial stem growth with positive strong correlation (Fig.2, $p<0.0001$, $\rho= 0.88$). Both radial stem growth and radial root growth were significantly correlated with both mean soil and air temperatures, and mean solar irradiance of the month preceding growth (Table.1). However, significantly strong negative correlations were found between both radial stem and radial root growth and precipitation of the month preceding growth ($p<0.001$, $\rho= -0.82$).

Table 1: Spearman correlations between both radial stem growth and radial root growth and mean soil and air temperatures (°C), mean soil volumetric water content (%), precipitation (mm) and mean solar irradiance (kw/m^2) of the month preceding the growth.

Growth	p-value	ρ
Radial stem		
SoilT	<0.001	0.57 **
AirT	<0.001	0.70 ***
θ	0.03	0.1 *
Precipitation	<0.001	-0.80 ***
Solar irradiance	<0.001	0.72 ***
Radial root		
SoilT	<0.001	0.54 **
AirT	<0.001	0.55 **

θ	n.s.	
Precipitation	<0.001	-0.82 ***
Solar irradiance	<0.001	0.53 **

3.3 Root elongation rate (RER)

Mean RER for growing roots differed significantly between soil depths over the year (Fig.3, p=0.001). Mean RER was significantly higher at a depth of 0.85-1.7 m (3.2 ±2.64 mm day^{-1}) than at 0.0-0.85 m (1.33±1.4 mm day^{-1}) and 4.0-4.7 m (1.43 ±1.59 mm day^{-1}) depths only during aerial late growing season, respectively. However, no significant differences were found in mean RER between depths during the aerial dormant season. Shallow fine roots (0.0-0.85 m) continued to grow almost all the year around and peaked to maximal rate in June and August. While at the second soil layer (0.85-1.7 m), budburst preceded root growth by 69 days. RER of this soil layer peaked two months later than the upper soil layer (0.0-0.85 m) reaching to maximal rate in August and October and then dropped down sharply to zero in December. However, small peaks in growth rate occurred during the dormant and early growing seasons at the two upper soil layers. At the third soil layer (2.0-3.3 m), root growth preceded the budburst by 31 days, peaked two months earlier than the second soil layer (0.85-1.7 m) in April or June before decreasing and then peaking to a higher extent in September, and dropped to zero in January. However, mean RER at the deeper layer (4.0-4.7 m), started 94 days after the budburst, peaked one month later than the third soil layer (2.0-3.3) to maximal rate in July before decreasing and then peaking to a higher extent in December, and dropping to zero in February (Fig.4). The first peaks of root growth in the upper two layers were higher than the second peaks of growth. However, at the two deeper soil layers (2.0-3.3 m, 4.0-4.7 m), the second peaks of root growth were higher than the first peaks of growth and root growth in these layers was delayed, resulting in the second peaks of growth by 2 and 4 months after the first peak compared to the two upper soil layers (one month).

Mean RER was significantly higher during aerial late growing season compared to the aerial dormant season ($p=0.03$) with much less root growth recorded in the early aerial growing season. A significant effect (Fig.5, $p=0.003$) of root diameter classes was found on mean RER for shallowly growing roots only. However, differences were found between the classes 0-1 mm and both 1-2 mm ($p=0.04$) and 2-5 mm ($p=0.009$) classes (Fig.5). Positive correlations were found between mean RER of growing roots and mean soil temperature of the month preceding the growth at both (0.0-0.85 m) ($p=0.04$, $\rho=0.27$) and (0.85-1.7 m) ($p=0.04$, $\rho=0.40$) soil layers only (Fig.6). However, mean RER was not correlated with either soil and air temperatures or θ during late aerial growing season. However, mean RER was significantly and positively correlated with mean solar irradiance of the month preceding growth (Fig.7, $p= 0.02$, $\rho=0.39$) at the uppermost soil layer (0.0-0.85 m) during late aerial growing season only. However, mean RER at deeper soil layers was not significantly correlated with any climatic factors.

3.4 Comparing above and belowground phenology

Leaf phenology and fine root growth were not synchronous at any soil depth. Root growth at both 0.0-0.85 m and 2.0-3.3 m soil layers peaked two month later than budburst. However, root growth at 0.85-1.7 m peaked four months later than budburst. However, root growth at a depth of 4.0-4.7 m peaked three months later than budburst.

Root growth reached the maximal rate later than budburst by 2, 4, 5, and 8 months at the four soil depths (0.0-0.85m, 0.85-1.7m, 2.0-3.3m, 4.0-4.7 m), respectively.

The timing of fine root growth at the two upper soil layers (0.0-0.85 m and 0.85-1.7 m) was synchronous with that of both radial stem growth and radial root growth during the late growing season only. However, the growth patterns were antagonistic, with peaks of fine root growth inversed to those of both radial stem growth and radial root growth during the late growing season. Whereas, fine root growth at depths of 2.0-3.3 m and 4.0-4.7 m followed similar patterns to those of radial stem growth and radial root growth during the late aerial growing season. In 2015, roots did not stop growing over the year in topsoil layer (0.0-0.85 m), and the length of the root growing season was higher at depths of 2.0-3.3 m (9 months) than at 0.85-1.7 m (6 months) and 4.0-4.7 m (6 months).

4. Discussion

Climatic factors effects on root elongation rate

Mean RER for growing roots over the entire year was correlated with mean soil temperature in the two upper soil depths only. However, when we focused on RER during the aerial late growing season only, we showed that no significant relationship existed between RER and soil / air temperature, or with θ, at any depth. These results are surprising and contrary to the findings of (Germon et al 2016) who studied walnut trees at the same stand. The absence of correlations of RER with any climatic factors is in conflict with other studies which indicate that air and soil temperatures are the prominent factors driving RER (Gill & Jackson 2000), (Misra 1999), (Hendricks et al 2006), (Mao et al 2013a), (McCormack & Guo 2014), especially during the growing season in temperate climates. Our results are also contradictory with those indicating soil water content as a driver of RER, e.g. Joslin et al, (2001), studying oak species (*Quercus prinus* L. *Quercus alba* L.) in subtropical climates and Joslin et al (2001); Olesinski et al (2011) studying *Abies balsamea* L in a temperate climate (Joslin et al 2001) (Olesinski et al 2011). In our study, soil temperatures for all soil depths never decreased below 5°C or above 23.5°C during the entire study. The significant relationship with soil temperature only at the two upper soil layers throughout the year is likely because the topsoil layers are more subjected to seasonal variations of aboveground conditions. These layers are usually humid in the winter and dry in the summer in a Mediterranean climate, contrary to deeper layers where soil is buffered against variations in environmental conditions (Waisel et al 2002). The possible reason for the absence of correlations of RER with any climatic factors during the aerial late growing season, is that temperatures were moderate during this period at all soil depths and ranged from 13-23.4°C, a temperature range considered favorable for walnut trees (Mohamed et al, 2016, unpublished data). Moreover, the sum of precipitations during the late aerial growing season (8 months) was 1053.2 mm, suggesting the absence of either anaerobic conditions or inadequate water conditions for growth. However, shallow fine roots only (0.0-0.85 m depth) were correlated with mean solar irradiance during late aerial growing season, as also found by (Fitter et al 1998) studying two grassland types along an altitudinal gradient. This result is also in accordance with other studies on e.g *Q. prinus* and *Q. alba* growing in a subtropical climate (Joslin et al 2001) Joslin et al (2001) found that root elongation intensity was influenced by the phenology index and not by soil temperature. This result could be interpreted in terms of carbon assimilation by photosynthesis, as solar irradiance provides energy to the metabolic processes in the tree; this energy contributes to many physiological processes depending on environmental conditions, e.g. photosynthesis, evaporation of different organs and also transpiration (Mahmoud & El-Gindy 2016). The energy needed to produce photosynthates (i.e. sucrose and starch) depends on the number of photons coming from solar irradiance (Amthor 2010), thus more energy will assimilate more CO_2 resulting in more sucrose and starch. The fraction of incident solar radiation intercepted depends on the total leaf area (Amthor 2010). In our study, the increase in RER with the increase of solar irradiance for shallowly growing roots (0.0-0.85 m) may be explained by the increase of leaf surface area during the leaf expansion phase.

In deeper soil layers, where roots are more proximal and hence need more time to receive photosynthates, we suppose that RER for growing roots was controlled by a relatively slower process of phloem transport rather than by any environmental factors.

In our study, the timing of root growth peaks differed between soil layers and was delayed with increasing soil depth. Shallow roots (0.0-0.85 m) continued growing almost all the year and peaked in June (leaf expansion phase) and August (the end of leaf expansion and total canopy cover). This result is in accordance with other studies, e.g. (Bauerle et al 2008) recorded winter root growth of *Vitis* spp. in a Mediterranean climate. Carbon fueling root growth during dormant and early spring seasons may come from non-structural carbohydrates (NSC) accumulated during the aerial growing season (Najar et al 2014), especially in deciduous trees, where photosynthesis does not occur during the dormant aerial season (Radville et al 2016). Our results suggest that the peaks of root growth observed were a trade-off between competing plant sinks (e.g., leaf expansion, radial stem growth, radial root growth and fine root elongation) during the growing season as stored carbohydrates were allocated to respiration and growth (Radville et al 2016), (Comas et al 2005). We suppose that fresh supplies of photosynthates were more important for leaf, radial root and stem growth, as well as and energy supply for shallow roots. All these sinks are more proximal to the source of NSC than deeper and distal fine roots, which peaked earlier than proximal organs. The maximal fine root elongation, and radial stem and root growth, took place during the late aerial growing season. As the main control of C allocation between roots and shoots is thought related to sink activity (Friend et al 1994), the decrease in fine root elongation in the two upper soil layers in July and August, respectively, may be due to non-structural carbohydrates (NSC) being used for radial stem and root growth as also suggested by (Endrulat et al 2016) studying *Abies alba*. These authors found that shoots did not supply roots with high amounts of photosynthate in the spring due to the investment in shoot growth, while in the summer and autumn; shoots allocated relatively high amounts of NSC to fine roots. However, another possible reason for the delay in growth peaks at the two deeper soil layers may be due to (i) deeper soil layers are slower to warm in spring and may retain moisture from both winter soil recharge and the water table (Radville et al 2016), thus the subsequent cambial activity in these roots would take longer to occur (ii) as soil water content increased significantly with increasing soil depth. We suppose that trees will promote deeper root growth in the period where temperatures are higher and where upper soil layers are drier (summer months), to improve water and nutrient uptake, demonstrating a positive feedback from developing roots to shoots (Friend et al 1994). The two deeper soil layers had root growth during October and December than earlier in the year, possibly because the newly formed fine roots in autumn received higher amounts of NSC (Endrulat et al 2016).

In our study, both radial stem and root growth were correlated with climatic factors (soil and air temperatures, precipitation and solar irradiance) as also found in previous studies (e.g. (Oribe et al 2001) studying *Abies sachalinensis,* who reported that cambial reactivation in spring was highly dependent on temperatures. However, radial stem and root growth were more strongly and negatively correlated with the sum of precipitation of the month preceding the growth. This finding is in accordance with that of (Schöngart et al 2002), studying deciduous and semi-deciduous species in Amazon floodplain forests, who also found a strong negative correlation between the monthly radial increment rates of stem and the mean water level of the same month, indicating a strong impact of the flood-pulse on tree development with low diameter increments at high water level. Such a negative correlation with the sum of precipitation in our study is likely because our study site is in a Mediterranean climate characterized with many thunderstorms during late summer and autumn (late growing season in our study), resulting in a rise in water table and occasional flooding. Flooding causes anaerobic conditions for roots, leading to a reduction of root activity and a water deficit in the crown, hence resulting in a cambial dormancy in both stem and root radial growth, and influencing the formation of annual rings in the wood by reducing the increment of both sinks (Schöngart et al 2002).

Our results demonstrated a significant positive correlation between RER and root diameter classes in shallow fine roots only. The possible reason for this result may be that the incorporation of C as structural C or as starch into the roots is strongly dependent on the root diameter (Endrulat et al, 2016), and the topsoil (0.0-0.85 m) had a higher number of roots in different classes of diameter compared to other deeper soil layers in our study. Thus, the influence of class of diameter was more evident for shallow roots. The high number of roots in the topsoil horizon is possibly due to competition with annual crops, stimulating quick initiation of more and finer roots in topsoil horizons in early spring, the period where crop roots were still present (May-June), and thus gaining access to limited resources before soil becomes drier in the topsoil and warmer in the deeper layers (Eissenstat & Caldwell 1988). Nevertheless, this finding is in conflict with our results presented earlier in this thesis previous (Mohamed et al, 2016, results not published) where we did not observe any differences between RER in different diameter classes. However, this result is in accordance with other observations by previous authors (e.g. by Germon et al, 2016 using minirhizotrons to quantify walnut trees growing in the same stand during the two first year of minirhizotrons installation). These observations about root proportion may also be due to the differences in the sample size analyzed (e.g. surface of observation, observed time scale or the number of roots per study) or the method used. In our previous study, we used only the rhizotron method (0.74 m^2 of soil profile surface) to quantify shallow root elongation. However, in this study, we combined observations from rhizotrons and minirhizotrons (1.58 m^2 of soil profile surface in topsoil layer), and by so doing we increased the sample size. In our previous study, the observation started later and the period of observation was shorter than the period in the current study (by 9 months), due to flood damage on rhizotrons, thus we may have missed the onset of root growth. For example, (McCormack et al 2015) studying temperate tree species found that root growth preceded leaf growth, but in another study with a shorter period of observations on the same plants, they found root production to peak after major leaf expansion (McCormack et al 2014). As the sample size increases, the margin of error decreases, thus increasing the sample size will provide more accurate results and will give us a better understating of the events occurring.

Comparing above and belowground phenology

In our study, the timing of root growth was asynchronous with that of budburst at all soil depths. However, root growth at the two upper soil layers (0.0-0.85 m and 0.85-1.7 m) was synchronous with both stem and root radial growth, but only during the late aerial growing season. The spring flush occurred several months after budburst (2-4 months) depending on the soil depth. Both budburst and root emergence are very sensitive to local temperatures (Du & Fang 2014, McCormack et al 2014), (Comas et al 2005), thus a rapid increase in air temperature in April would stimulate the budburst quickly as well as reactivate cambial activity in the stem. While soil buffered against the rapid changes in air temperature, therefore the subsequent cambial activity in fine roots and structural roots would take longer time to occur with increasing the soil depth (e.g. soil temperature decreased significantly by increasing soil depth during late growing season in our study) resulting flushes of root growth after budburst (Pregitzer et al 2000). Both stem and root radial growth flushed later than budburst, possibly fueled by NSC supply during the early spring. Radial growth peaked in parallel and inversely with fine root elongation at the upper two soil layers during the late growing season, when photosynthesis was occurring and NSC levels were higher for supporting growth.

5. Conclusion

Because of the difficulty in making measurements of seasonal root growth, especially deep root growth, firm conclusions about how climate influences root and shoot phenology is difficult to obtain. Our results highlight that shoot (leaf and stem) and root (structural roots, shallow fine roots and deep fine roots) respond differently to climatic factors and that they are under different controls throughout the year. Environmental cues may be part of a signal for initial root production (Fitter et al 1999) and may govern the number of root initiation. However, subsequent growth appears to be regulated by endogenous factors possibly more linked to photosynthesis supply (Comas et al 2005). Our results conclude that if other factors are equal, stored NSC is very important during root initiation in deciduous species where photosynthesis does not occur in the early growing season. While subsequent growth may be almost entirely dependent on current (not stored) supplies of photosynthates and carbon allocation processes, we suggest that depending on the period of year, activity of the sink (for shallow roots only) and the proximity of the source to the sink as well as vascular connections between them (for deeper roots), are important regulators of growth. Hormones may play an essential role in driving C allocation processes as enzymes regulate starch-sucrose partitioning and are influenced by plant hormones (Friend et al 1994). Hormones produced in a given organ can also regulate the phenology of distal organs. Hence, more focus is needed on how the internal dynamics of trees interact with climatic factors to influence whole plant phenology. Furthermore, in our study as we have found that root growth responds to solar irradiance rather than other climatic factors, then day length and cloudiness seems to be an important variable to consider in future studies as both factors influence the carbon flux to the soil.

6. Figures

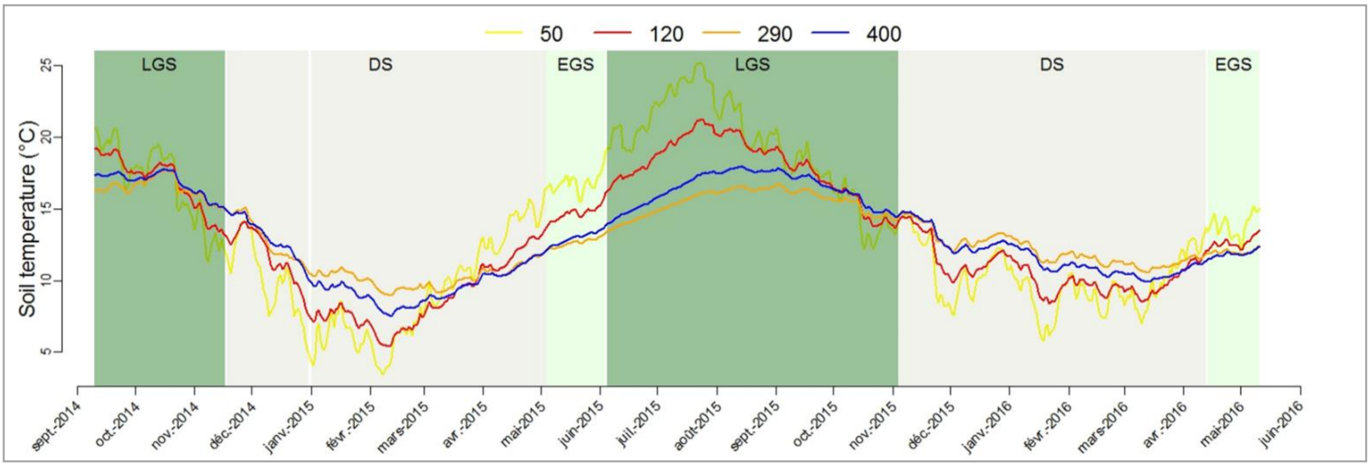


Fig. 1: Soil temperatures at the four soil layers, 50 cm depth (yellow line), 120 cm depth (red line), 290 cm depth (orange line), 400 cm depth (blue line) over the observation period from September 2014 to June 2016 at the Mediterranean field site. Different background colors correspond to different phenological periods: “LGS” is late growing season (green) “EGS” is early growing season (light green), “DS” is dormant season (gray).

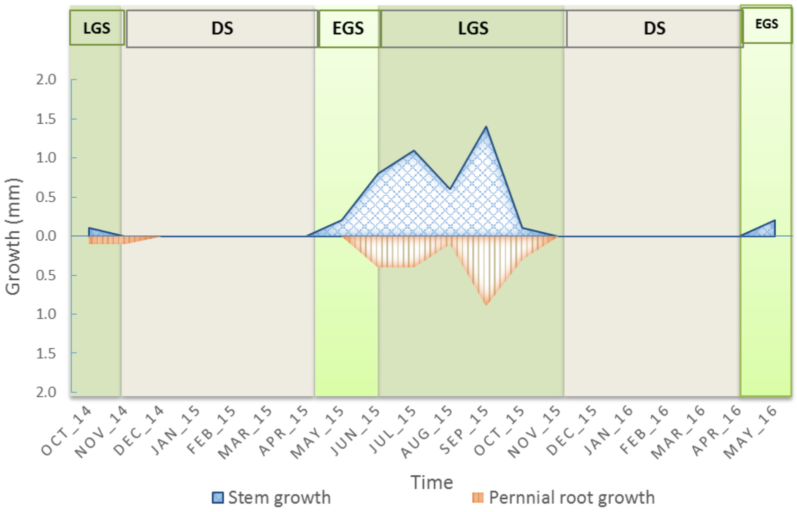


Fig. 2: Mean radial stem growth (blue area) and mean radial root growth (orange area) of walnut trees at the Mediterranean field site over the observation period from October 2014 to May 2016. Different background colors correspond to different phenological periods: “LGS” is late growing season (green) “EGS” is early growing season (light green), “DS” is dormant season (gray).

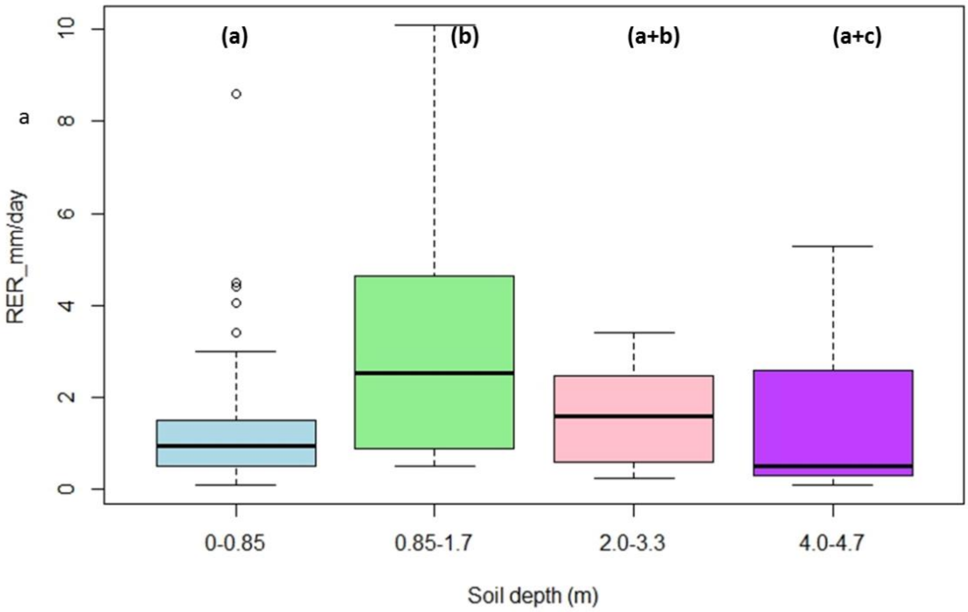


Fig. 3: Comparison of mean RER (mm/day) of the growing roots between soil depths (m) during the entire observation period. Different letters above the boxplots indicate statistically significant differences ($p < 0.05$) between methods.

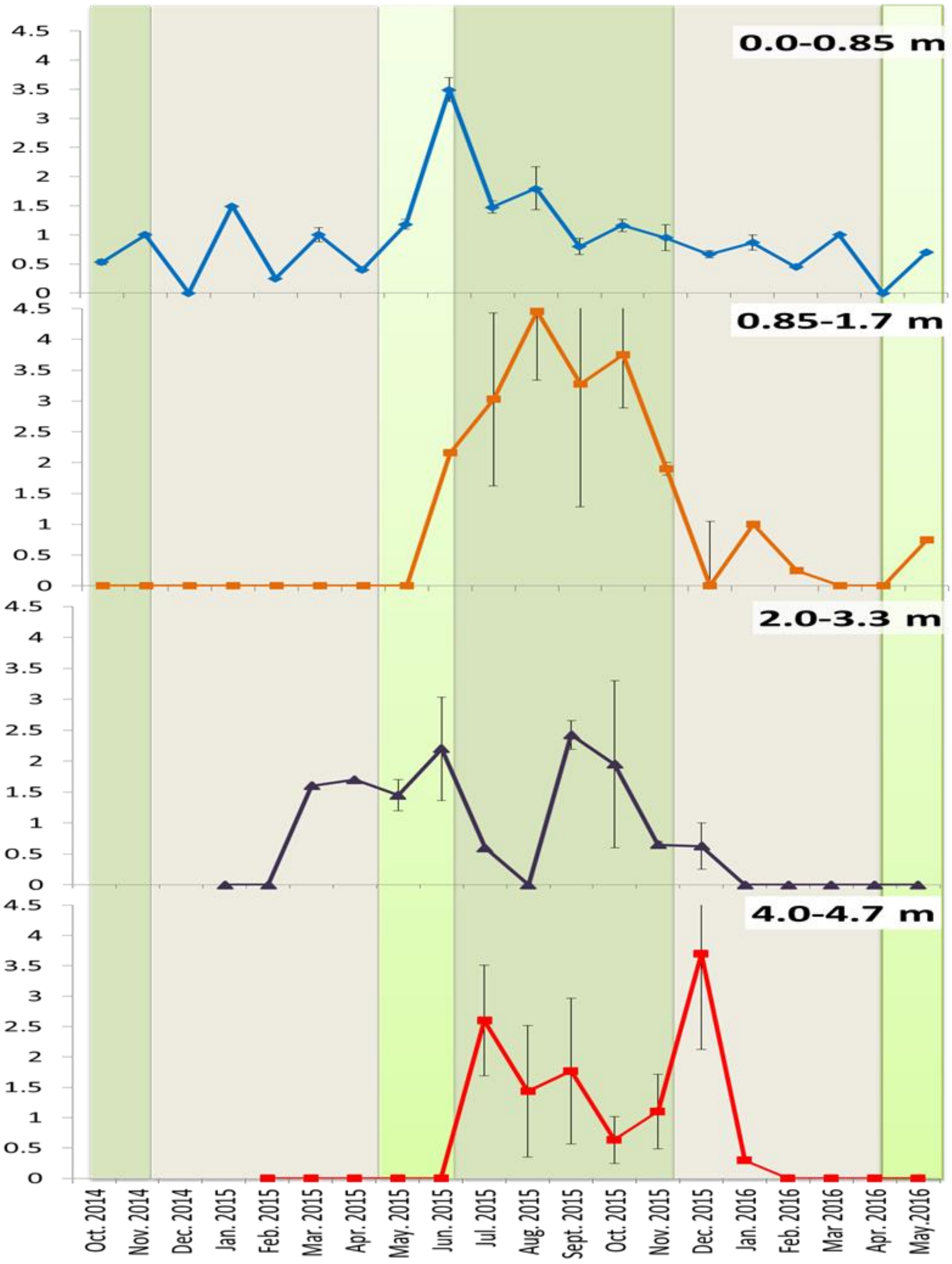


Fig. 4: Mean root elongation rate at 0.0-0.85 m (blue line), 0.85-1.7 m (orange line), 2.0-3.3 m (violate line) and 4.0-4.7 m (red line) at a) oceanic, b) Mediterranean field sites over the observation period from October 2014 to June 2016. Different background colors correspond to different phenological periods: "LGS" is late growing season (green) "EGS" is early growing season (light green), "DS" is dormant season (gray). Missing data/ flood damage is shown with a dotted line over the dashed curves.

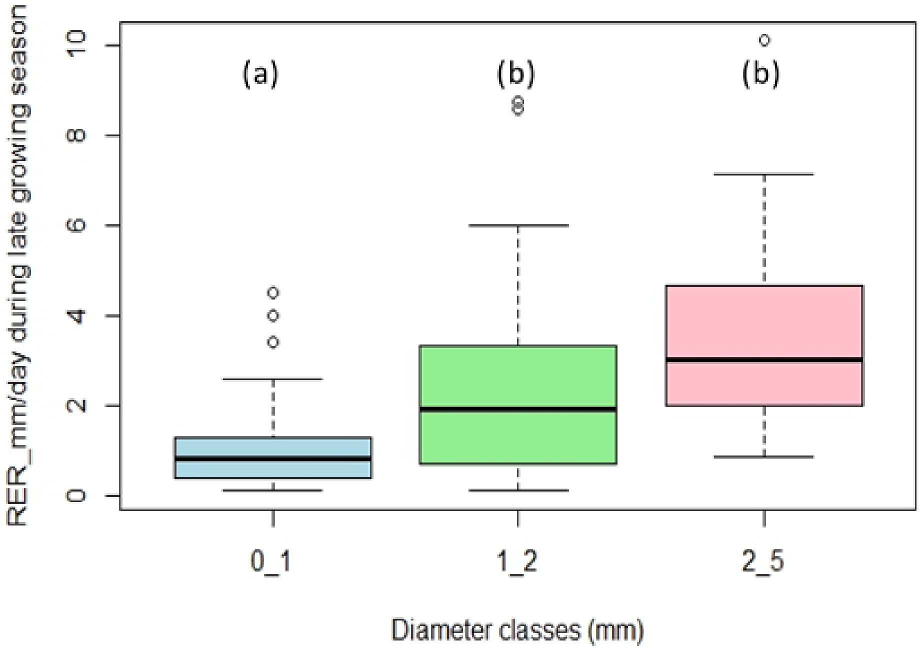


Fig. 5: Comparison of the mean RER between diameter classes (mm) of the growing roots at (0.0-0.85 m) depth during the late growing season. Different letters above the boxplots indicate statistically significant differences ($p < 0.05$) between methods.

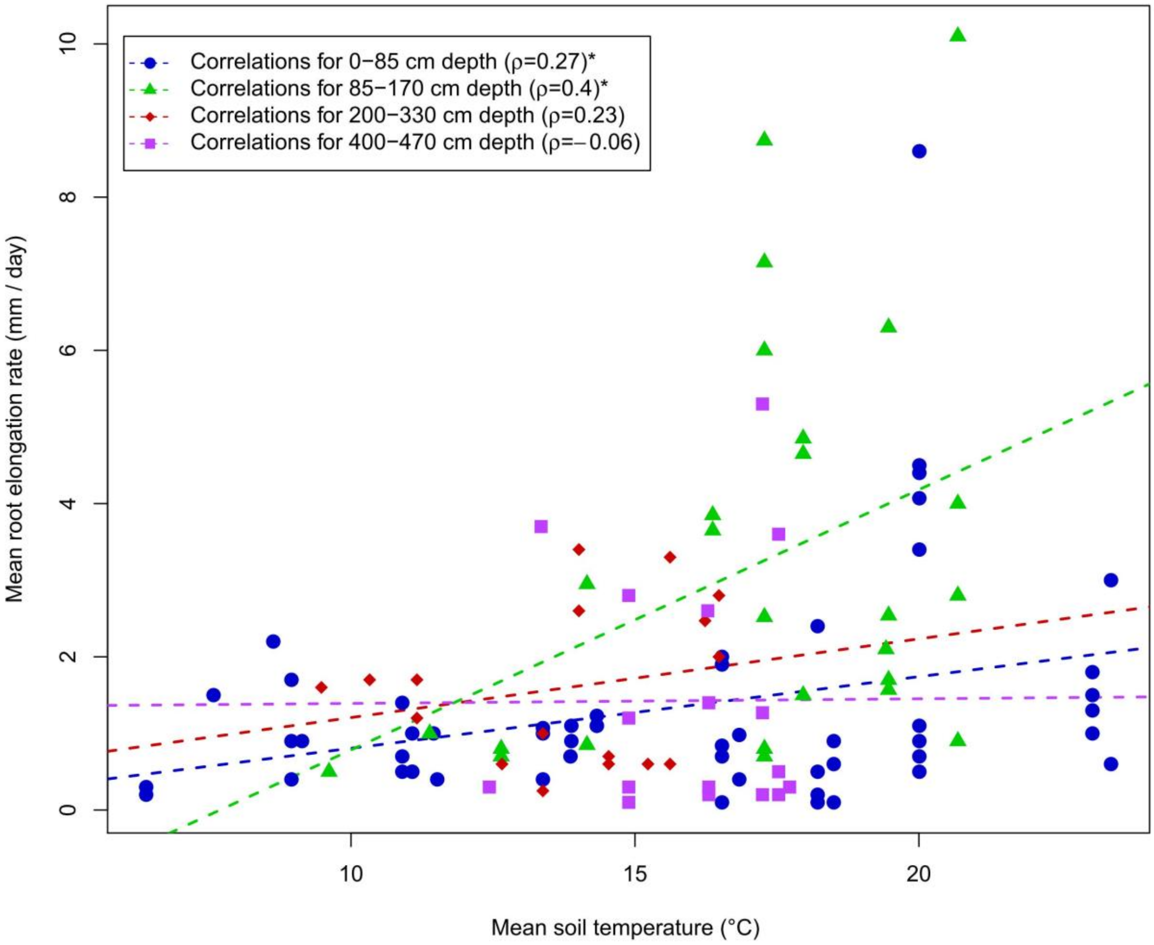


Fig. 6: Correlations between mean root elongation rate (mm/day) and mean soil temperature (°C) during the entire observation study at 0.0-0.85 m (blue circles), 0.85-1.7 m depth (green triangles), 2.0-3.3 m depth (red diamonds) and 4.0-4.7 m depth (purple squares).

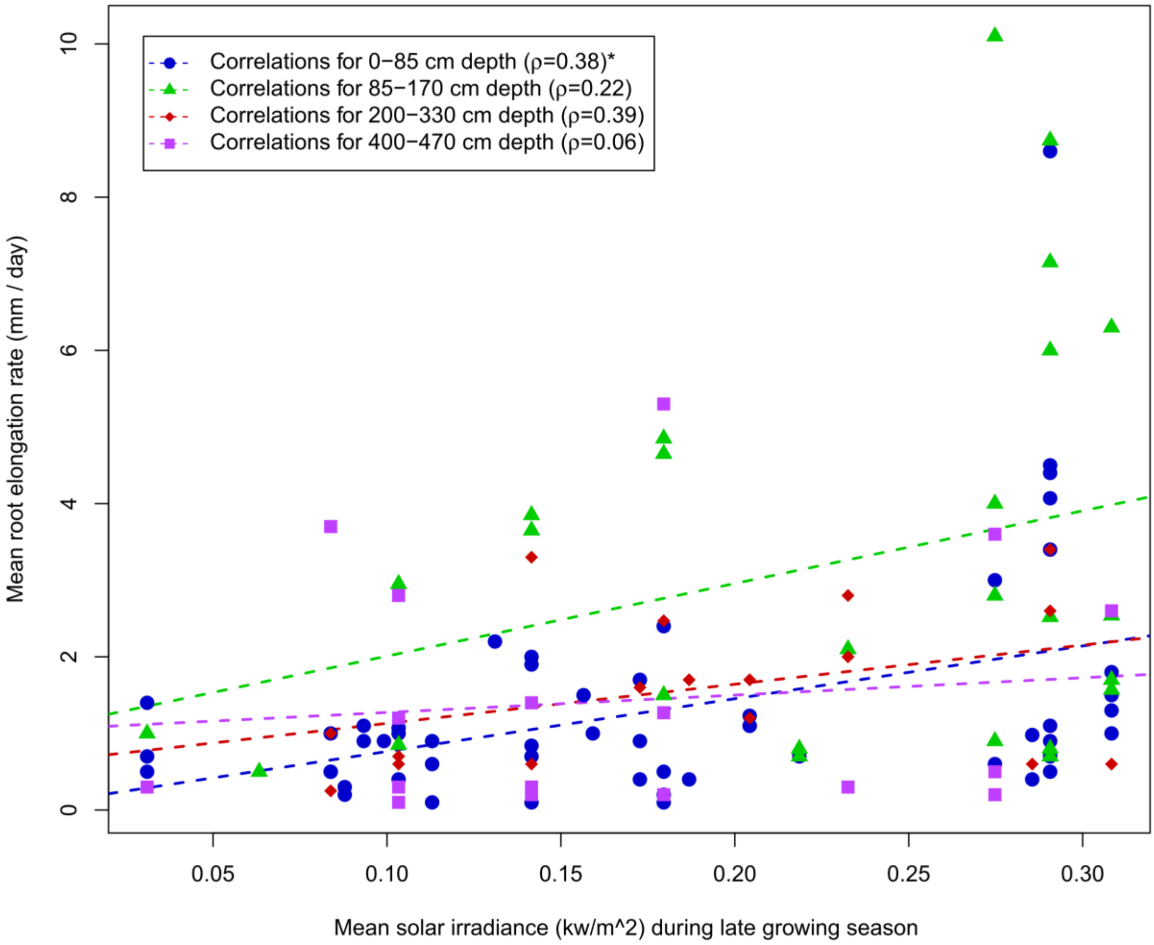
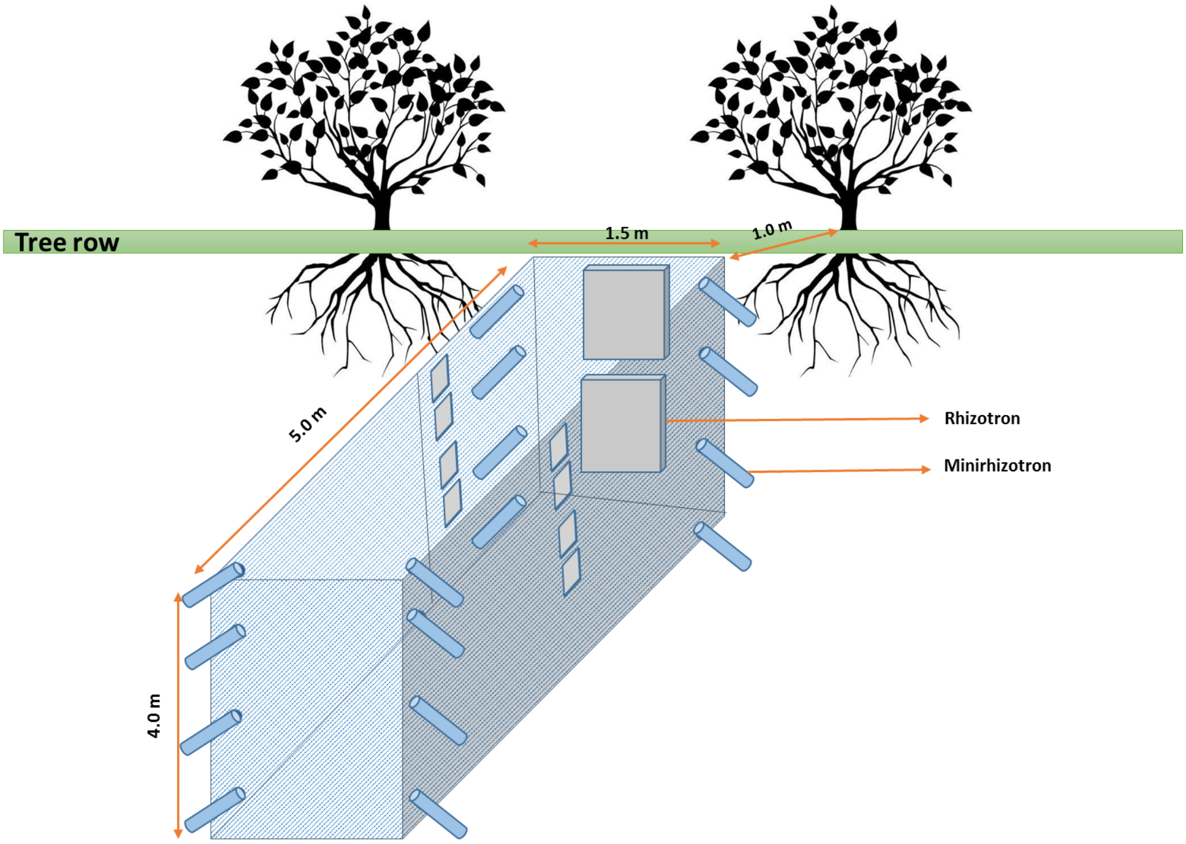


Fig. 7: Correlations (for late growing season only) between mean root elongation rate (mm day^{-1}) and mean solar irradiance (kw/m^2) at 0.0-0.85 m (blue circles), 0.85-1.7 m depth (green triangles), 2.0-3.3 m depth (red diamonds) and 4.0-4.7 m depth (purple squares).

7. Appendix

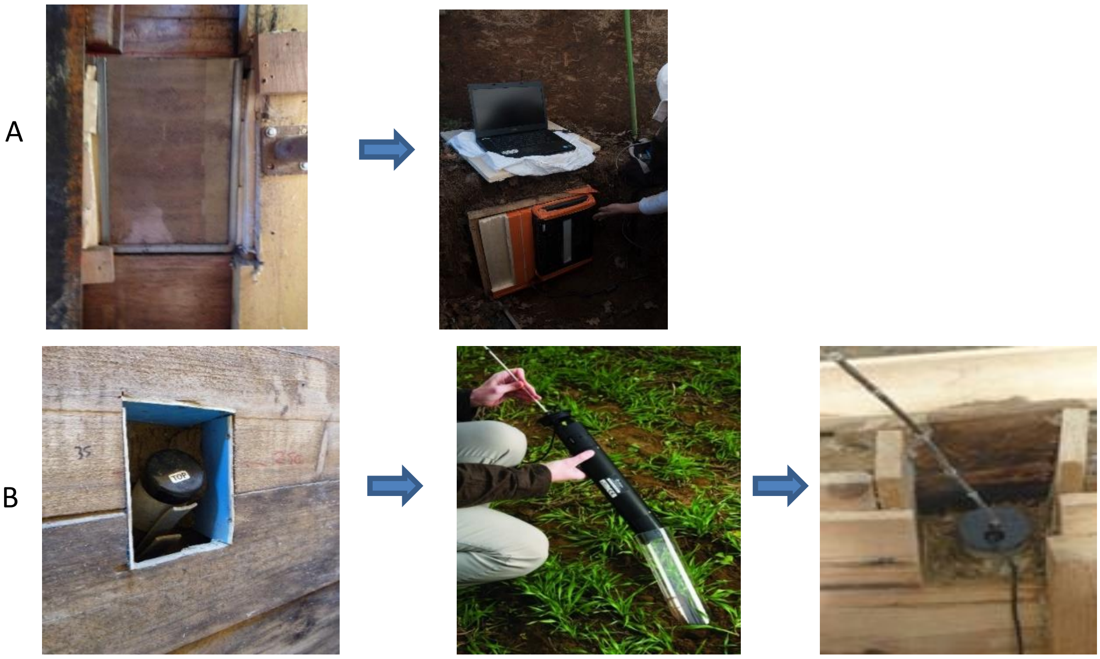
Appendix 1: Agroforestry system at Mediterranean climate



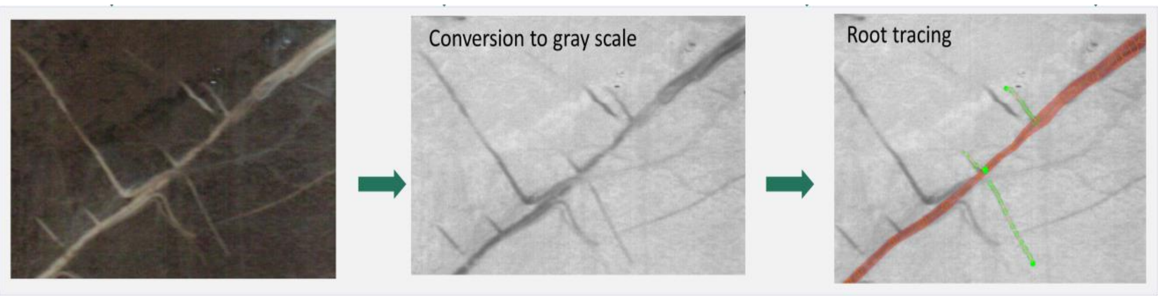
Appendix2: The big pit with all rhizotrons and minirhizotrons installed

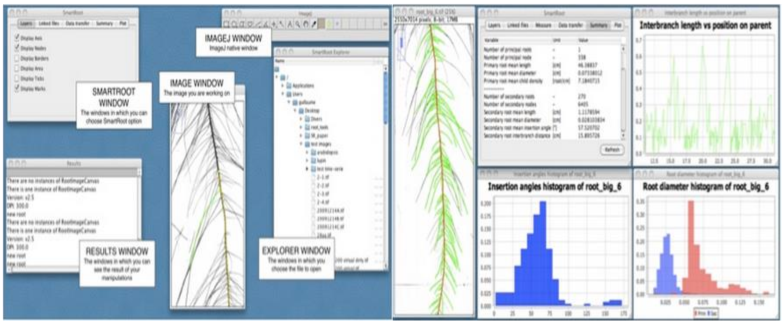
Appendix 3: The big pit covered with a metallic roof

Appendix. 4: Flood damages



Appendix. 5: A) Flatbed scanner (Epson Perfection V370 flatbed scanner) and B) circular scanner (CI-600, CID, USA) to monitor root growth in both rhizotron and minirhizotron, respectively.





Root analysis

SmartRoot is a semi automated image-analysis software -independent freeware based on ImageJ to streamline the quantitative analysis of root growth and architecture of complex root systems -independent freeware based on Image.(Lobet et al).

Appendix. 6 : SmartRoot

Appendix. 7: Dendrometers installed on the structural roots and the stem of walnut tree

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Chapter V: General discussion

1 Root methodological problems

Due to the important role of roots in ecosystem functions and nutrient and carbon cycling, (Gill & Jackson 2000, Vogt et al 1995) studies on root growth have been numerous over the last decade and a significant progress in evaluating root morphology has been observed. However, research remains challenging and costly especially in the natural environment. In the first part of this thesis, we focused on opportunities to simplify the measurements of root morphological and architectural properties without which we cannot progress in the quantifying of root dynamics. Many nondestructive methods, such as rhizotrons and minirhizotrons have been developed to overcome some of the limitations of observing root systems in the natural environment. Here, we compared five methods of root observations (smartphone scanner application, flatbed scanner, handheld scanner, time-lapse camera and tracing onto transparent sheet) when using *in situ* rhizotrons and we evaluated each method with regard to accuracy, time and cost, to determine which method gave the best results. Our results demonstrate that all five methods can be used to monitor root length. However, if accurate measurements of root diameter are required, we suggest using one of the scanners, due to the overestimation obtained in root diameter when using time-lapse camera or manual tracing. With regard to the quality of images, time, and cost criteria, the smartphone scanning application was found to be performing best overall when considering all criteria. It provided images of high resolution with minimal deformation. Moreover, the application was free and did not need any accessories. Data acquisition took a short time and treatment was carried out directly in the application without spending time to treat images in other softwares before analyzing images with the Smartroot software. In addition, smartphones are widespread and have a wide community of developers. We showed that the flatbed scanner method also is rapid, easy to use, inexpensive and give a high quality of images, also found by (Dannuora et al, 2008; Dong et al, 2003; Adu et al, 2014) (Adu et al 2014, Dong et al 2003) (Dannoura et al 2008), but it has many accessories so it is not easily transportable. Furthermore, flatbed scanners cannot scan a large surface area of a rhizotron at one time, therefore many scans are needed for one surface area of 50x50 cm, and thus more time was needed to merge images before analysis. Automatic flatbed scanners have not been developed yet, therefore, the scanner needs a power supply in the field to acquire images over a long period in the field. Similar to the flatbed scanner, the handheld scanner was rapid, portable and gave good quality images, also found by Pan et al, 1998, but also needed many images to cover the surface of the rhizotron so more time is needed for merging images manually before analyzing them. While scanners cannot automatically acquire images in the field over a long period, our results showed that the time-lapse camera can be left in place for several months without any manual intervention and it is relatively inexpensive. In contrast, the quality of images was poor and a certain amount of reflection occurs from the flash, leading to poor quality images. Additionally, the lenses of the time-lapse camera are less developed and have less optical resolution to those of smartphones camera, leading to blurred photos taken by the time-lapse camera, especially in belowground conditions (e.g. humidity). Thus, the SmartRoot software was unable to distinguish and detect correctly the border of roots, because the software estimates the dimeter of the root by measuring diagonally nodes along the root, and so an overestimation of root diameter occurred. The diameter of roots taken by the time-lapse camera can be corrected if we have simultaneous measurements with a flatbed scanner on a number of roots and we can therefore estimate the real diameter by correlating the two measurements. Nevertheless, our results showed that the main advantage of a time-lapse camera was in examining the influence of the circadian clock on root growth in the field, as the most recent studies on circadian clock were performed on the laboratory due to the non-availability of a fully automated method to measure root growth constantly in the field. To the best of our knowledge, the smartphone scanning application and the time-lapse cameras have never been used to monitor root growth in the field, even though they are inexpensive and easy to use, compared to more sophisticate such as minirhizotron scanners.

2 Root growth and mortality drivers

Environmental conditions vary widely between climates over the year, and can have different impacts on plant function and production. In the second part of this thesis, we focused on the impact of climatic variables and root morphological traits on root growth and mortality of walnut trees growing in three agroforests using *in situ* rhizotrons along a climatic gradient in France. Two methods were adopted to acquire root images through rhizotrons: time-lapse camera (oceanic site) and flatbed scanner (continental and Mediterranean sites). Our results marked one distinct flush of root growth, initiation and mortality during the aerial growing season, regardless of the climate, and negligible growth occurred during dormant seasons. This result confirms previous observations performed by Contador et al, 2015 on walnut trees in the Mediterranean climate. In this study and for all climates mean soil temperatures did not fall below 3°C or above 21°C during the entire study. Contrary to the finding of Heninger White (1974) (Heninger & White 1974) that highest root growth of deciduous trees occurred when soil temperatures were above 20°C, in our study maximal RER occurred when the soil temperature was within the range 14-17°C. No root growth was recorded when the soil temperature was below 5°C, as also demonstrated by Alvarez-Uria and Koerner, 2007) in temperate environments. Our results showed that factors driving root production and mortality were not the same between climates (Table 1). Surprisingly, in our study, mean RER was positively correlated with both mean soil and air temperatures at the oceanic and continental sites, but not at the Mediterranean site where Germon et al (2016) found a highly significant and positive correlation between mean RER and mean soil temperature for the same stand of walnut hybrids. Whereas, when we considered the late growing season (June to November) where the high rate of was elongation found, mean RER was driven by soil temperature at the oceanic site only. This result confirms previous observations performed by Misra (1999) (Misra 1999), studying *Eucalyptus* seedlings in an oceanic climate and King et al, (1999) (King et al 1999) studying *Populus tremuloides* trees in a temperate climate. However, at the Mediterranean site, where the climate is characterized by dry summers, mean RER was driven by mean soil water potential, contrary to the two other sites where the summers were wetter and cooler, suggesting that soil water was limiting for root growth in superficial layers at the Mediterranean climate, as also found by Misson et al, (2006) (Misson et al 2006) in *Pinus ponderosa* in a Mediterranean climate. Contrary to previous observations (e.g. Steinaker et al (2010) (Steinaker et al 2010) studying several woody plants and Fukuzawa et al, (2013) (Fukuzawa et al 2013) studying *Abies sachalinensis* in a continental climate), climatic variables did not drive mean RER at the continental site. Mean RER was significantly higher at both oceanic and Mediterranean climates compared to the continental site, while the continental site had more roots and laterals roots than the two other sites. The possible reasons for this result may be that the soil at the continental site was more acidic (pH=4.5), and so heavy metals were mobilized in the soil and passed into the soil solution and were taken up by roots, reducing the growth of individual roots and increasing slightly lateral root number, as indicated by Kahle, (1993). Kahle, (1993) studying *Fagus sylvatica*, *Acer rubrum and Pinus resinosa* seedlings found that root systems became more branched and dense due to damage of root tips by heavy metals. Moreover, the continental site was phosphorous deficient, and the low level of phosphorous in the soil may increase the heavy metal uptake by tree roots. The soil at the continental site was not deep, and rocks were present at a depth of 1.5 m making a physical barrier and impeding root elongation. Surprisingly and contrary to the observations of previous authors (Germon et al, 2016) working on walnut cultivars studying *Populus deltoides Bartr* (Kern et al 2004), mean RER was not related to root diameter.

Drivers of root initiation differed between sites with soil temperatures more linked to root initiation at both continental and Mediterranean sites only. However, root initiation was linked to air temperature at both oceanic and Mediterranean sites. These relationships were positive at the oceanic site but negative at both Mediterranean and continental sites, possibly because mean annual soil temperature at the oceanic site was significantly lower (11°C) and so correlations were more clear, with an optimal value of 14-17°C for root growth. This result confirms also observations found by Mao et al, (2013) studing *Picea abies* and *Abies alba* in a montane forest. Although the drivers of root mortality still remain unknown for the oceanic and Mediterranean sites, mortality was linked to both soil temperature and water potential at the continental site, confirming the finding of Harris et al, (1995) (Harris et al 1995) studying *Acer saccharum* in a moderate continental climate. Mean root initiation (mRIQ) was higher at both continental and Mediterranean sites compared to the oceanic site. Mean RMQ did not differ significantly between sites and was highest during the late growing season at the three sites. We consider the peak of mortality as a consequence of a trade-off between competing plant sinks to balance carbohydrate availability.

Table. 1 Drivers of root growth and mortality during the late growing season at the three climates sites

Root indicator	RER	RIQ	RMQ
Drivers			
Oceanic	soilT***+airT**+ψ**	airT*	unknown
Continental	unknown	soilT***+airT*	soilT*+ ψ **
Mediterranean	ψ *	SoilT***+airT**	unknown
Potential drivers			
Oceanic	-	-	NSC, hormones
Continental	pH, P, soil	-	-
Mediterranean	-	-	NSC, hormones

3 Shoot and root relationships

Our results show that the timing of root production depends on climate. At all sites the timing of root growth lagged behind that of budburst and the spring root flush occurred several weeks later than budburst depending on climate. As both budburst and root occurrence are very sensitive to local temperatures (Tierney & Fahey 2002; Du & Fang 2014), a rapid increase in air temperature in April/May would stimulate budburst quickly. Soil warmed up slower compared to air temperatures in spring (Polgar & Primack 2011), therefore the subsequent cambial activity in roots would take longer to occur, and root flushes will usually occur after bud burst (Pregitzer et al 2000; Steinaker & Wilson 2008). In deciduous trees, stored NSC plays a major role in driving root growth in the early spring before leaf unfolding, because they do not photosynthesize early in the season (e.g. minor root elongation occurred during aerial growing season at all sites in our study). Once leaves have unfolded in these trees, concurrent NSC investment by plant organs will control the peaks of their growth. In our study, the decrease in fine root elongation observed in August (oceanic and continental sites) and July (Mediterranean site) may be due to NSC being used for radial growth and fruit production. NSC production from photosynthesis would then increase during the summer, fueling a second root flush, before leaf senescence in November. The decrease in photosynthetic rates at the end of the growing season would result in less NSC being available for radial growth, which decreases rapidly in September – October (Du & Fang 2014) (Abramoff & Finzi 2015; Radville et al 2016). We suggest that stored carbohydrates play a smaller role in controlling the timing of peak of root growth when leaves are already present. For example in evergreen trees, root growth may continue fairly constantly throughout the growing season without synchronizing with foliage growth, because these species have the potential to photosynthesize all year round. Additionally, plant growth hormones regulate all aspects of vascular differentiation in plants (Du & Fang 2014; Aloni 2013). These hormones can be influenced by the variation in temperatures, soil water content and nutrient availability. For example, plants that are nutrient deficient may delay the peak of root growth to late spring or summer to compensate for low NSC stores (López-Bucio et al 2003; Radville et al 2016). Moreover, trees in competition with herbs or crops may also advance the timing of peak root growth to access resources faster than their neighbors (Eissenstat & Caldwell 1988).

The peaks of both root growth and mortality occurred during the late growing season regardless of climate. Therefore, root production and mortality of walnut trees is inherently programmed to occur during the late growing season (June to November) with significantly less production in the aerial dormant season.

4 Root survivorship

Another important indicator to consider for understanding root system performance, and which also provides a considerable amount of carbon and nitrogen to soil (Cox et al 1977) (Wells & Eissenstat 2001) was root survivorship. In our study, roots did not possess the same longevity at the three sites and longevity changed among phenological periods over the year. The effect of climate was clear on root longevity and roots at continental site lived longest. Walnuts trees at this site made thicker roots and root diameter was correlated to longevity. This result confirms also the observations of Anderson et al (2003) on *Vitis labruscana*. The possible reason is that roots in colder environments possess a longer lifespan compared to a warmer environment, because they have lower respiration rates (e.g. the continental site in our study during the dormant season), as also shown by McCormack and Guo (2014) and Burton et al, (2000) (McCormack & Guo 2014; Burton et al 2000). While the decrease in root longevity at higher temperatures (e.g. the Mediterranean site in our study) may be the result of increased metabolic activity, inducing a buildup of free radicals and therefore faster root aging (McCormack and Guo (2014). We also showed that roots in the 0-1 mm and 1-2 mm diameter classes lived for a significantly shorter period compared to those in the 2-5 mm diameter class at both continental and Mediterranean sites only. The possible reason for this result is that thicker roots have lower N concentration, lower surface area and higher C content and thus metabolic activity decreased resulting in longer-liver roots (McCormack et al, 2012, Guo et al, 2004, 2008) (Guo et al 2008, Luke McCormack et al 2012; Guo et al 2004). In our study, root longevity was also affected by root topological orders, one of the important factors influencing root traits. First order roots lived longer than lateral roots, as also shown by McCormack et al, (2012) studying 12 temperate tree species. We suggest that first order roots live longer than laterals because of the greater investment in their construction, while finer roots cost less to construct and thus can grow quickly and die quickly, when water and nutrient resources are low.

5 Deep fine root phenology

Endogenous and exogenous factors both have an impact on fine root growth. How these factors interact with each other or by which mechanism they control root growth is still less known. In the third part of this thesis, we focused on the impact of climatic variables on deep fine roots of walnut trees growing in alley cropping agroforestry system in a Mediterranean climate in France. We also concentrated on relating shoot phenology (leaf and stem) to root phenology (perennial root and shallow and deep fine roots) in this system. We used rihizotrons and minirhizotrons to monitor root elongation rate throughout the year by adopting two type of scanners to measure root systems through both rhizotrons and minirhizotrons (Flatbed scanner and circular scanner), respectively. Root elongation rate is an important indicator of root phenology. It is a consequence of both cell divisions on the meristem in the apex of root and cell elongation in elongation zone (Jijoon 2006). Root elongation rate can maintain whole plant function thanks to its plasticity in the soil matrix for water and nutrient uptake (Mao et al 2013). RER may vary between classes of diameter or branch order or soil depth (Germon et al 2016, Mao et al 2013). This variation in RER can affect tree's carbon allocation by influencing the rhythm of root respiration (Högberg et al 2002).

Recent works show that root phenology may differ by depth in some ecosystems and may be controlled by different factors (Germon et al 2016, Radville et al 2016a). Our results confirm these results and showed that mean root elongation rate (RER) differed between soil depth and did not flushed in the same time at all soil depth. This result also is in accordance with the finding of Germon et al.(2016) studying walnut trees for the same stand in Mediterranean climate. Although Germon et al. (2016) reported a positive correlations between RER and soil temperatures, our results surprisingly and contrary to their observations showed no relationships between RER and neither soil and air temperatures nor soil water content in any soil depth during late aerial growing season. This result is in conflict also with observations of other authors in Mediterranean climate studying *Juglans regia* L. (Contador et al 2015), *Pinus ponderosa* P., (Misson et al 2006), or *Quercus ilex* L. (Coll et al 2012, López et al 1998). However, in our study, RER of shallow fine roots only (0.0-0.85 m) was positively related to mean solar irradiance of the month preceding the growth. This result confirms the finding of et al. (1998) studying two grassland type along an altitudinal gradient and Fu et al. (2016a) studying deciduous shrubs in subtropical climate, but in contrast with the finding of Radville et al. (2016b) studying Arctic shrub-graminoid. A possible explanation in failing to find correlation with both temperature and soil water content may be because soil temperature was optimum for root growth during late growing season in our study and ranged between (13-23)°C as also we have shown in our previous study for walnut trees growing in different climates (unpublished data). Moreover, summer drought is a characteristic trait of Mediterranean climates and can limit primary production (López et al 1998), but as the sum of precipitation during late growing season in our study was (1050.2 mm), and as deeper soil layers can retain humidity from winter soil recharge because they are slower to warm

(Radville et al 2016a), we suggest an adequate water in the soil for supporting root growth, thus soil water content is not the main driver of growth.

The mechanism that allows roots to grow with increasing solar radiation may be explained by the amount of carbon produced during the photosynthesis and the carbon allocated in each plant organ. Assimilation of CO2 molecule to photosynthate (sucrose and starch) needs energy, which is controlled by the number of photons in the solar irradiance absorbing by leaves, therefore, influencing the quantity of photosynthate produced. As the fraction of solar irradiance intercepted by plant depends on the leaf area (Amthor 2010), we suppose that the increase in RER of shallow roots (0.0-0.85 m) with increasing solar irradiance during late growing season is related to an increase in photosynthate produced during photosynthesis, consequently to the increase in leaf surface area during leaf expansion phase. This response to solar radiation may differ between deciduous species and evergreen species (Fu et al 2016b), and may be because evergreen species have the potential to photosynthesize all year round, thus other factors such as variations in temperature or soil water content could be the main factors driving root growth in these species. For example, Fu et al. (2016b) studying the influence of shading on evergreen and deciduous shrubs in subtropical climates, showed that variations in the annual fine root production and turnover to shading were significant in deciduous species only, and that decreasing shading resulted increasing in root production in spring. In our study, spring root flush occurred several weeks (8-12 weeks) later than budburst depending on soil depth. Fine root phenology was asynchronous with leaf phenology in all soil depths. Roots can grow fairly and constantly throughout the year if no extremes climatic factors happens (i.e. the case of shallow roots in our study). Bauerle et al. (2008) also confirm this result by reporting a winter root growth of *Vitis* spp.in Mediterranean climate. In our study, peaks of growth were delayed with increasing of soil layers. The two upper soil layers (0.0-0.85 m and 0.85-1.7 m) peaked two and three months (June and July) later than budburst, before decreasing and then peaked another time to less extent two month later (August and September respectively). The two deeper soil layers (2.0-3.3 m, 4.0-4.7 m) peaked 3 and 4 months later than budburst before decreasing and then peaked another time to higher extent two and four months later (September and December, respectively). However, radial stem and root growth were synchronous with the two upper soil layers (0.0-0.85m and 0.85-1.7 m) during late growing season. We showed that both maximal radial stem growth and radial root growth were found during late growing season. Radial stem growth occurred after budburst and preceded radial root growth by one month.

The possible explanation of the differences in the timing of peaks between different soil layers and different plant organs may refer to a trade-off between competing plant sinks (e.g., leaf expansion, radial stem growth, radial root growth and fine roots elongation) as stored carbohydrates are allocated to respiration and growth (Comas et al 2005, Radville et al 2016a). This result confirms the finding of Abramoff & Finzi (2015) who reviewed that Mediterranean plants are driven by endogenous factors rather than exogenous factors. It was assumed that the main control of C allocation between roots and shoots is related to sink activity (Friend et al 1994). Thus we suppose the decrease in RER at the two upper soil layers in July and August may be due to non-structural carbohydrates (NSC) being used for radial growth of both stem and structural roots, as both radial stem and root growths were found in late growing season only. This result confirms the observation of Endrulat et al. (2016) on *Abies alba*, they indicated that shoot did not supply roots with high amounts of photosynthate in spring due to the investment in shoot growth while in summer and autumn; shoots allocated relatively a high amounts of (NSC) to fine roots, and this may play a key role in driving this mechanism. In deciduous species, carbon fueling root growth during dormant and early growing season may come from NSC accumulated during aerial growing season contrarily to evergreen species which has photosynthetic tissues round a year (Najar et al 2014). Moreover, deep soil layers are slower to warm because they buffered against the rapid increasing in air temperature, and as well as retain moisture from winter recharge more than superficial layers. Thus the subsequent cambial activity in these roots would take longer time to occur, and therefore occurred later than budburst rapidly stimulated by the increasing in air temperature (Radville et al 2016a). Furthermore, in deeper soil layers, where roots are more distant and hence need more time to receive photosynthate production, we suppose that root elongation rate for growing roots was controlled by a relatively slow process phloem transport rather than by any environmental factors. In addition, as soil water content increased significantly with increasing soil depth in our study, we suppose that tree may promote deeper roots in the drought period (summer in Mediterranean climate) to improve water and nutrients uptake confirming a positive feedback from developing roots to the developing shoot (Friend et al 1994) and this mechanism may be controlled by plant growth regulators (López-Bucio et al 2003).

It was assumed that newly fine roots formed in autumn contain highest carbon enrichment in the starch than those formed in spring (Endrulat et al 2016, Hansen et al 1996) which may explain our finding that the second peaks of growth in (September and December) had higher extent than the first peaks in the two deeper soil layers. Kuptz et al.(2011) studying beech (*Fagus sylvatica*) and spruce (*Picea abies*), also support this finding, and demonstrated that during spring only negligible amounts of new photosynthates enter the transfer pool for supporting shoot growth (leaf and stem). While during early summer, new photosynthates support directly the growth and transported to roots, and during late summer, new photosynthates supported storage pools, supplying growth.

Our result showed a positive correlation between class of diameter and mean RER of only shallow growing roots, supporting the finding of (Germon et al 2016) on walnut tree in the same stand in Mediterranean climate. However, surprisingly this result is in contrast of our finding in previous study on walnut trees in the same stand. The missing of correlations with deeper roots in this study and also in our previous study may be due to changing of method or of the sample size (e.g. surface of observations, observed time scale or number of growing roots included in the analysis). In our previous study, only rhizotrons were combined in the analysis with a surface of observation of (0.74m^2), while in this study we increased the surface of observation by combining both rhizotrons and minrhizotrons to (1.58 m^2 of soil profile surface in topsoil layer), and thus the number of roots entered to analysis was increased. In addition, the period of observation in our previous study was shorter than current study due to flood damage that we faced, and thus may resulting the missing of the onset of root growth. McCormack (2015) studying temperate tree species found that root growth preceded leaf growth in one study, but in another study with a shorter period of observations on the same plants, found root production to peak after major leaf expansion (McCormack et al 2014). We suggest that by increasing the surface of observation, we decrease the margin of error, and thus providing more accurate results.

General conclusion and perspectives

The inability to have firm conclusions about how global change factors will affect root dynamics or how changes in root dynamics might affect plant functioning or C cycling in soil is due to many problems: (i) methodological problems in measuring unseen root systems without disturbing the system. (ii) The difficulty to generalize this impact in the face of broad variability in responses among plant species, biomes and climates.

This study permitted us to answer three challenging questions concerning root research:

(i) We compared five techniques of root image acquisition when using *in situ* rhizotrons and evaluated each method. We propose using the inexpensive method of a smartphone scanner to acquire images when using *in situ* rhizotrons. Our results also highlighted that scanners and time-lapse cameras provide correct measurements of root elongation in the field. Time-lapse cameras overestimate root diameter but are useful for taking frequent images of root elongation in the field over several months, without any manual intervention. Our results call for further analyses to improve methodology, e.g. if future generations of smartphones could scan images and transfer data automatically. Developing time-lapse cameras with higher optical resolution or consensually to the optical resolution of smartphones should also be performed.

(ii) We also demonstrated relationships between fine root phenology (root elongation, initiation, mortality and survivorship) and shoot phenology (leaf phenology and stem radial growth) of walnut trees across three temperate agroforest systems during different periods throughout the year. Our results highlight that abiotic factors drive fine root production when they are limiting (e.g. soil water potential at Mediterranean site in our study and soil temperatures at both continental and oceanic site) and if they are not limiting, endogenous factors such as NSC and hormones may play major roles in driving root production (Fig. 1).

Once soil temperature is favorable for roots, and if there are no extremes of temperature throughout the year, then soil temperature is not the main driver of root growth and other limiting factors will drive root growth such as soil water availability (e.g. Mediterranean site in our study). We showed clear differences between shoot and root phenology. Root and leaf phenology was asynchronous at the three climates with the major pulse of root production during the late growing season, regardless of length of both aerial (6.0-7.5 months) and belowground (7.5-8.5 months) growing seasons, suggesting that hybrid walnut root production is inherently programmed to occur during the late growing season with significantly less production in the aerial dormant season in the three climates. However, our results show the influence of climate on the timing of peaks of root production during the late growing season (e.g. RER peaked in June (continental site), July (oceanic site) and August (Mediterranean site). Through a multi-covariate analysis of root survivorship (site, root diameter, root topology orders and phenological periods), we found that all these covariates were positively correlated with root survivorship. The effects of site and root diameter were the strongest predictors to root survivorship among these factors. Further analyses on the role of site conditions (altitude, topography, plant genotype) in determining tree responses to climate change should be performed. In addition, the seasonal phenology of trees is a main driver of C allocation from shoots to roots, thus further research is required to evaluate more precisely the relationships between the internal dynamics of tree carbon and nutrient resources and root phenology.

(iii) We focused deeper on relationships between root phenology (structural root and both shallow and deep roots) and shoot phenology (leaf and stem) of walnut trees at the Mediterranean climate throughout the year. Our results highlight that among all climatic factors, solar irradiance plays a prominent role in driving indirectly shallow fine root growth if other climatic factors are equal. We validated our hypothesis that the drivers of root growth differ between soil depths. While roots at topsoil layer (<1) was driven by abiotic factors (solar irradiance), deeper roots were likely driven by endogenous factors such as NSC allocation and hormones. Our results suggest that new photosynthates from spring mainly remain in the shoots to support the leaf and radial growth of stem, structural roots, and superficial fine roots before drought period, whereas photosynthates from summer and autumn are allocated in larger portions to the fine roots (especially deep roots) to improve water and nutrient uptake when conditions are not optimal in the upper soil layers, resulting in delay in peaks of deep fine root versus peaks of shallow root growth. We confirmed also that fine roots were not synchronous in any soil depth with leaf phenology, while fine roots in the two upper soil layers (<2m) only were synchronous with radial stem and radial root growth. More focus is needed on how the internal process of plants (hormones and carbon allocation) interacts with climatic factors to influence whole plant phenology. Furthermore, the length of day and solar radiations seem to be important variables to consider in future studies on root phenology as both factors influence the carbon flux to the soil. Further studies should also give specific attention to the sample size to obtain a more accurate understating of on how plant belowground phenology responds to climate change.

This study permitted us to quantify root dynamics of walnut trees growing in agroforest systems along a climatic gradient and through different soil depths. Choosing a similar ecosystem with similar cultivars under different climates and soil depths clarified better our understanding of how climate variability influences root dynamics and therefore how root system performance of the same cultivars changes when the environment changes. Knowledge about such dynamics in agroforest systems can help to gain insight into the factors driving soil carbon sequestration in further developments of climate-smart agricultural projects.

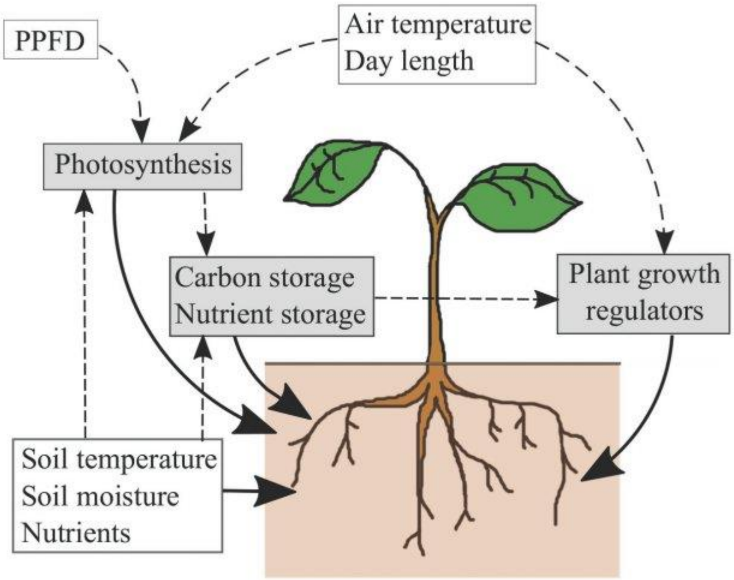

Fig. 1. Potential controls over root phenology. Solid lines indicate direct controls and dashed lines indicate indirect controls on root phenology. Gray boxes represent endogenous controls; and white boxes represent exogenous controls. PPFD is photosynthetically active flux density.

(Extracted from Radville et al, 2016)

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Résumé: Objectifs, résultats, conclusions générales

Dans cette thèse, nous posons plusieurs questions:

- Quelle est la meilleure technique pour l'acquisition d'image des racines en ce qui concerne la qualité de l'image, du temps et du coût lors de l'utilisation de rhizotrons in situ?
- Qu'est-ce qui gouverne la dynamique racinaire le long d'un gradient latitudinal?
- Est-ce que les facteurs qui contrôlent la croissance et la mortalité des racines sont les mêmes?
- Est-ce que la partie souterraine et aérienne est synchrone ?
- La croissance aérienne et racinaire sont-ils sous les mêmes contrôles ?
- Est-ce que la phénologie racinaire diffère en fonction de la profondeur ?

Les hypothèses principales:

- ❖ La partie racinaire et aérienne sont asynchrones indépendamment du climat ou de la profondeur du sol.
- ❖ Les facteurs qui contrôlent la croissance racinaire et la mortalité racinaire ne sont pas les mêmes entre les climats.
- ❖ Le diamètre de la racine est lié à l'élongation racinaire et diffère en fonction des périodes phénologiques.
- ❖ La longévité racinaire diffère par rapport aux traits morphologiques à travers des climats
- ❖ La partie racinaire et la partie aérienne sont sous différents contrôles.
- ❖ Les moteurs de croissance racinaire diffèrent en fonction de la profondeur.

Les objectifs:

- Quantifier la dynamique racinaire en utilisant des rhizotrons et des minirhizotrons au cours de l'année.
- Comparez diverses techniques d'acquisition d'image des racines afin de choisir la meilleure pour chaque site expérimental.
- Examiner l'influence de la température de l'air et du sol, le potentiel hydrique du sol, Irradiation solaire, la teneur en eau utile sur la dynamique racinaire en fonction de différentes périodes phénologiques.
- Examiner l'effet du site et les différents traits morphologiques de la racine sur la survie des racines au cours de l'année.
- Relier la phénologie de la partie aérienne (feuille et tronc) à la phénologie racinaire (racines de structures, racines fines superficielles et profondes).

Les approches générales et les sites d'étude

Dans cette thèse, nous avons étudié les processus souterrains en lien avec la phénologie aérienne des noyers (*Juglans* L.) en systèmes agroforestiers en France. Afin de vérifier nos hypothèses et répondre à nos questions, nous avons mené nos expériences sur le terrain, ce qui nous permet de mieux comprendre comment la variabilité climatique influence la dynamique racinaire des arbres lorsque les racines sont dans leur environnement naturel. Pour quantifier la croissance et la mortalité des racines (chapitre III) et la croissance racinaire par classe de la profondeur du sol (chapitre IV), nous avons utilisé des rhizotrons et des minrhizotrons. Les rhizotrons et les minirhizotrons sont des moyens rentables et non destructifs pour observer la croissance racinaire des plantes in situ. L'avantage des rhizotrons par rapport aux minirhizotrons est qu'ils offrent une plus grande surface mesurable et un meilleur contact entre le sol et les racines, cependant l'estimation de l'élongation racinaire peut être mieux surveillée avec les rhizotrons.

Les résultats généreux

Les résultats ont montré l'indépendance des phénologies aériennes et racinaires, mais pas la synchronisation des croissances racinaires et radiales du tronc. Le principal facteur influençant la dynamique racinaire est la température du sol, et à moindre mesure l'humidité du sol avec des effets contrastés selon le climat. Les réponses des dynamiques racinaires aux variables climatiques restent propres à chaque site, avec un fort impact de l'ordre topologique. Un troisième objectif était d'étudier la phénologie des racines d'horizons 'profond' (2-4m), et leur synchronicité vis-à-vis d'autres parties de l'arbre. Nous avons montré que la production de racines profondes se faisait de manière asynchrone des parties aériennes et racinaires superficielles, quasi indépendamment des fluctuations climatiques, avec des pics de croissance jusqu'en période hivernale.

Conclusion générale et perspectives

L'incapacité d'avoir des conclusions fermes sur la façon dont les facteurs de changement global auront une influence sur la dynamique racinaire ou comment les changements de la dynamique racinaire pourraient affecter le fonctionnement de la plante ou le cycle du carbone dans le sol est en raison de nombreux problèmes: (i) des problèmes méthodologiques afin de mesurer le système racinaire, la partie cachée de la plante, sans perturber le système . (ii) La difficulté de généraliser cet impact face à une large variabilité des réponses entre les espèces, les biomes et les climats.

Cette étude nous a permis de répondre à trois questions difficiles concernant la recherche racinaire:

(I) Nous avons comparé cinq techniques d'acquisition d'image des racines via rhizotrons in situ en évaluant chaque méthode. Nous proposons d'utiliser la méthode smartphone, la moins coûteuse pour acquérir des images via rhizotrons in situ. Nos résultats ont également mis en évidence que les scanners et les time-lapse caméras fournissent des mesures correctes de l’élongation racinaire sur le terrain. Nous avons trouvé que les time-lapse caméras surestiment le diamètre de la racine mais sont utiles pour prendre des images fréquentes des racines sur le terrain pendant plusieurs mois, sans aucune intervention manuelle. Nos résultats exigent des analyses complémentaires pour améliorer la méthodologie, par ex. Si les générations futures de smartphones pouvaient numériser des images et transférer des données automatiquement. Ainsi que le développement de time-lapse caméras avec une résolution optique plus élevée ou bien consensuellement à la résolution optique des smartphones pourraient développer la méthode.

(ii) Nous avons également démontré des relations entre la phénologie racinaire (l’élongation, l’initiation, la mortalité et la survie) et la phénologie aérienne (la phénologie des feuilles et croissance radiale du tronc) des noyers en trois systèmes agroforestiers tempérés au cours de différentes périodes au cours de l'année. Nos résultats mettent en évidence que les facteurs abiotiques contrôlent la production racinaire lorsqu'ils sont limites (par exemple, le potentiel hydrique du sol dans le site méditerranéen dans notre étude et les températures du sol à la fois sur les sites continental et océanique) et s'ils ne sont pas limites, ce sont des facteurs endogènes comme le NSC et les hormones qui peuvent jouer un rôle majeur dans la conduite de la production racinaire. Une fois que la température du sol est favorable pour les racines et s'il n'y a pas de température extrême au cours de l'année, la température du sol n'est pas le moteur principal de la croissance racinaire et que d'autres facteurs peuvent limiter la croissance racinaire comme la disponibilité de l'eau dans le sol (Ex. Site méditerranéen). Nous avons également montré des différences entre la phénologie aérienne et racinaire. La phénologie racinaire était synchrone avec celle des feuilles pour les trois climats avec une majorité de production durant la saison où les feuilles sont fonctionnelles, quelle que soit la longueur des saisons de croissance aérienne (6,0-7,5 mois) et souterraine (7,5 à 8,5 mois). Ce qui suggère que la production de noyer hybride est intrinsèquement programmée pour se produire pendant la période où les feuilles sont fonctionnelles, avec une production nettement négligeable durant la dormance aérienne dans les trois climats. Cependant, nos résultats montrent l'influence du climat sur le moment des pics de production racinaire pendant la saison où les feuilles sont fonctionnelles (Ex. Le RER a atteint son pic en juin (site continental), en juillet (site océanique) et en août (site méditerranéen).

Grâce à une analyse multi-covariables de la survie des racines (site, diamètre de la racine, ordre des topologies de la racine et les périodes phénologiques), nous avons constaté une corrélation positive entre toutes ces covariables et la survie des racines. Les effets du diamètre des racines et du site ont été les prédicteurs les plus forts pour la survie des racines parmi tous ces facteurs.

D'autres analyses sur le rôle des conditions du site (altitude, topographie, génotype végétal) dans la détermination des réponses des arbres aux changements climatiques devraient être effectuées. En outre, la phénologie saisonnière des arbres est un facteur principal de l'allocation de C de la partie aérienne vers les racines, donc d'autres recherches sont nécessaires afin d’évaluer plus précisément les relations entre la dynamique interne du carbone des arbres et les ressources nutritives et la phénologie racinaire.

(iii) Nous nous sommes concentrés sur les relations entre la phénologie racinaire (les racines de structures et les racines superficielles et profondes) et la phénologie aérienne (les feuilles et le tronc) des noyers au climat méditerranéen au cours de l'année. Nos résultats mettent en évidence que, parmi tous les facteurs climatiques, l'irradiation solaire joue indirectement un rôle important dans la conduite d'une croissance des racines fines superficielles si d'autres facteurs climatiques sont égaux. Nous avons validé notre hypothèse selon laquelle les facteurs qui gouvernent la croissance racinaire diffèrent en fonction de la profondeur du sol. Alors que les racines de la couche superficielle (<1m) étaient influencées par des facteurs abiotiques (irradiation solaire), les racines plus profondes étaient probablement influencées par des facteurs endogènes tels que l'allocation NSC et les hormones. Nos résultats suggèrent que les nouvelles photosynthètes du printemps restent principalement dans la partie aérienne pour soutenir la croissance de la feuille et de la croissance radiale du tronc, des racines structurelles et des racines fines superficielles avant la période de sécheresse, tandis que les photosynthètes de l'été et de l'automne sont attribués en grandes portions aux racines fines (en particulier les racines profondes) pour améliorer l'absorption de l'eau et des nutriments lorsque les conditions ne sont pas optimales dans les couches supérieures du sol, ce qui entraîne un retard dans les pics des racines fines profondes par rapport aux pics de croissance des racines superficielles.

Nous avons également confirmé que les racines fines profondes n'étaient pas synchrones avec la phénologie des feuilles, alors que les racines fines dans les deux premières couches supérieures du sol (<2m) n'étaient que synchrones avec la croissance radiale du tronc et des racines de structures.

Il est nécessaire de prêter plus d’attention sur la manière dont le processus interne des plantes (hormones et allocation de carbone) interagit avec les facteurs climatiques pour influencer la phénologie de la plante entière. En outre, la durée de jour et les rayonnements solaires semblent être des variables importantes à considérer dans les études à venir sur la phénologie racinaire, car les deux facteurs peuvent influencer le flux de carbone dans le sol. D'autres études devraient également accorder une attention particulière à la taille de l'échantillon prise afin d’obtenir une compréhension plus précise de la façon dont la phénologie souterraine des plantes répond aux changements climatiques.

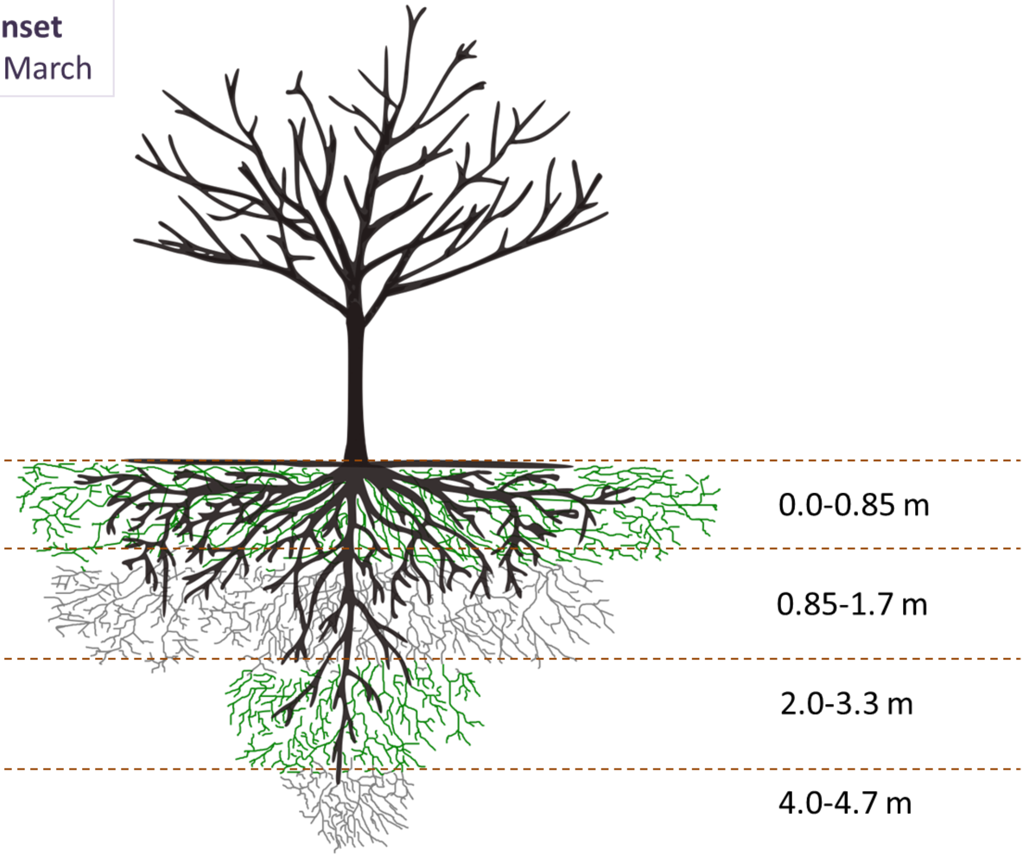
Cette étude nous a permis de quantifier la dynamique racinaire des noyers qui poussent dans des systèmes agroforestiers le long d'un gradient climatique et à travers des différentes profondeurs du sol. Le choix d'un écosystème similaire avec des cultivars similaires sous différents climats et différentes classe de profondeurs du sol a clarifié notre compréhension de la façon dont la variabilité climatique influence la dynamique racinaire et, par conséquent, comment la performance du système racinaire des mêmes cultivars change lorsque l'environnement change. La connaissance de cette dynamique dans les systèmes agroforestiers peut aider à non seulement mieux comprendre les facteurs qui gouvernent la séquestration du carbone dans le sol, mais aussi à l'amélioration des modèles écophysiologiques.

Conclusion

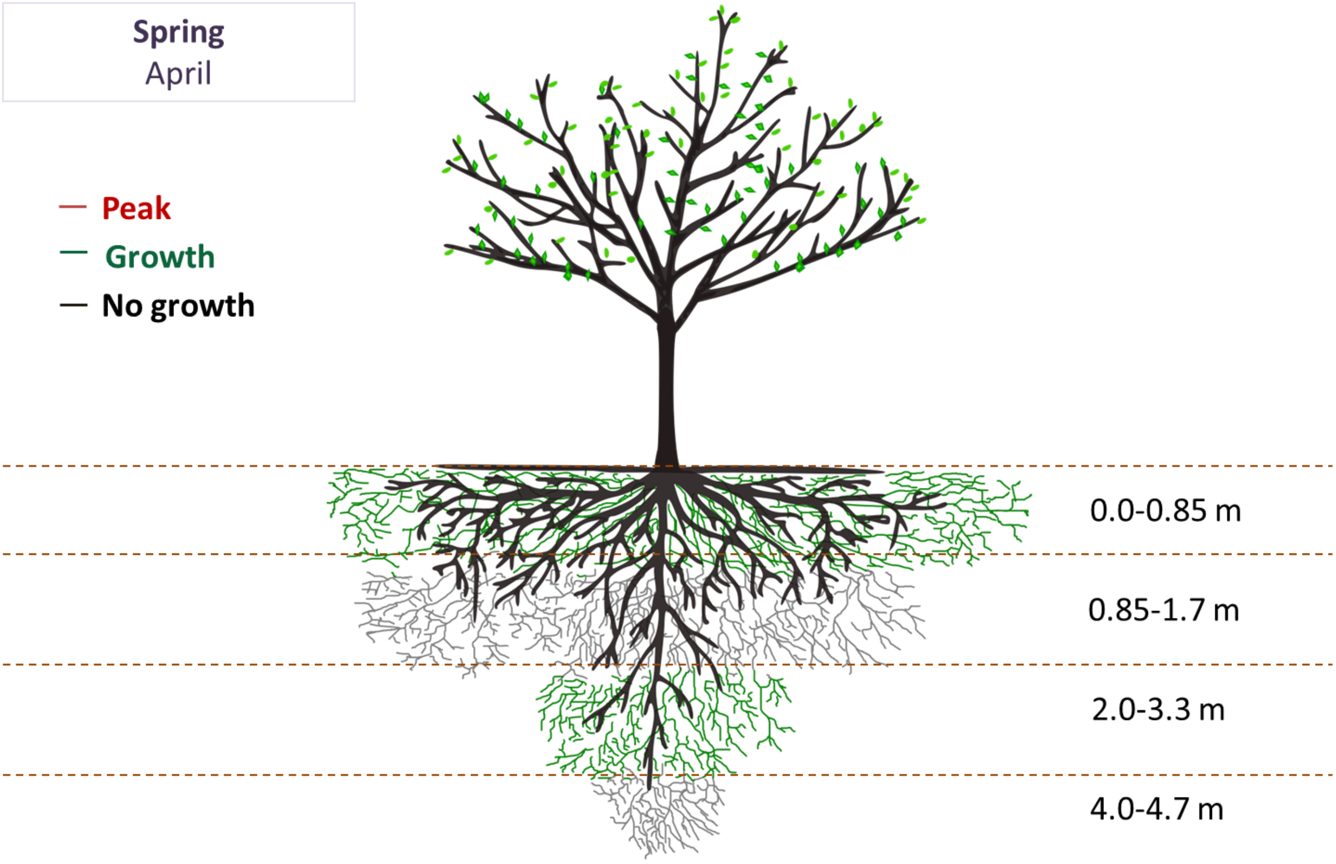
Deeper roots continue growing in the dormant season

Winter & Spring onset
January, February & March

— **Peak**
— **Growth**
— **No growth**

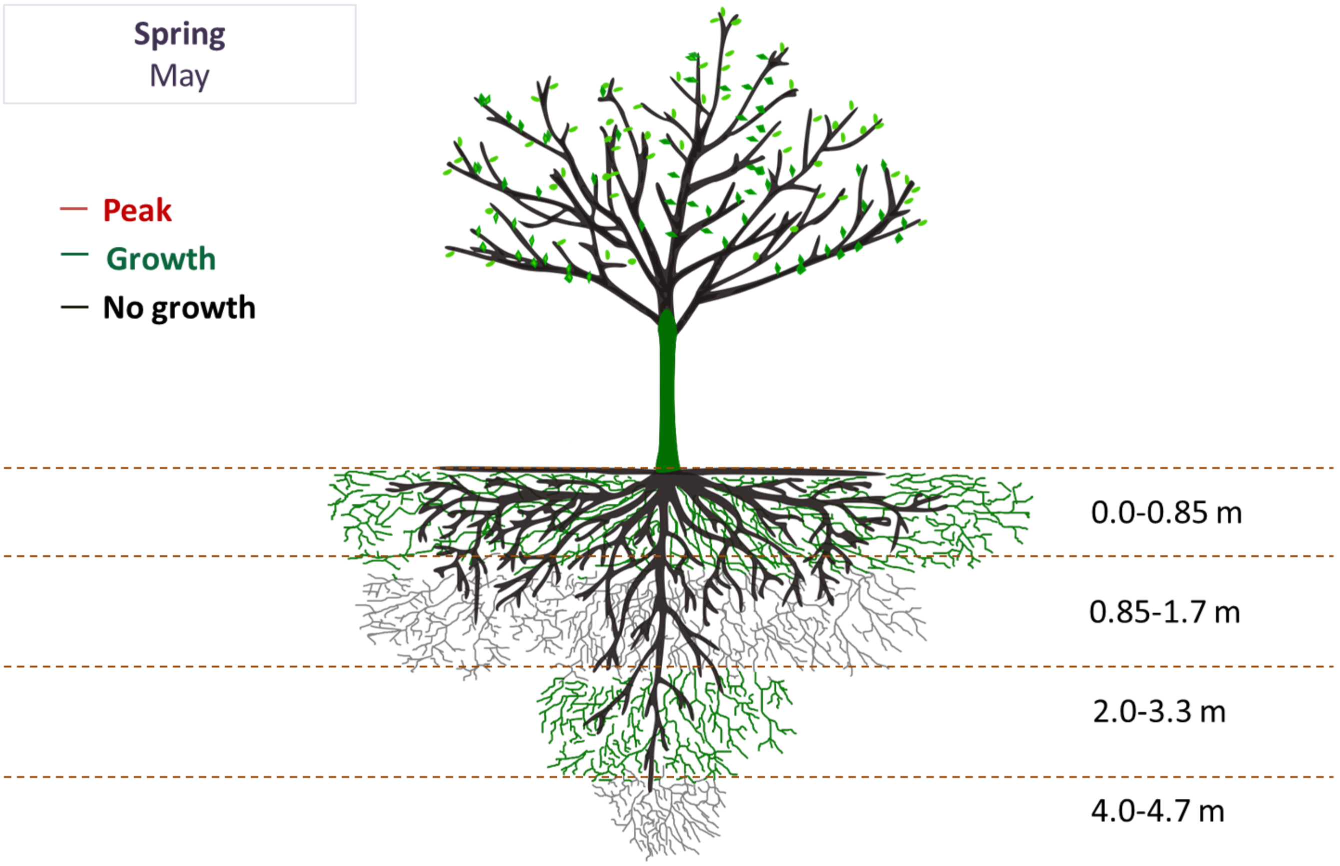


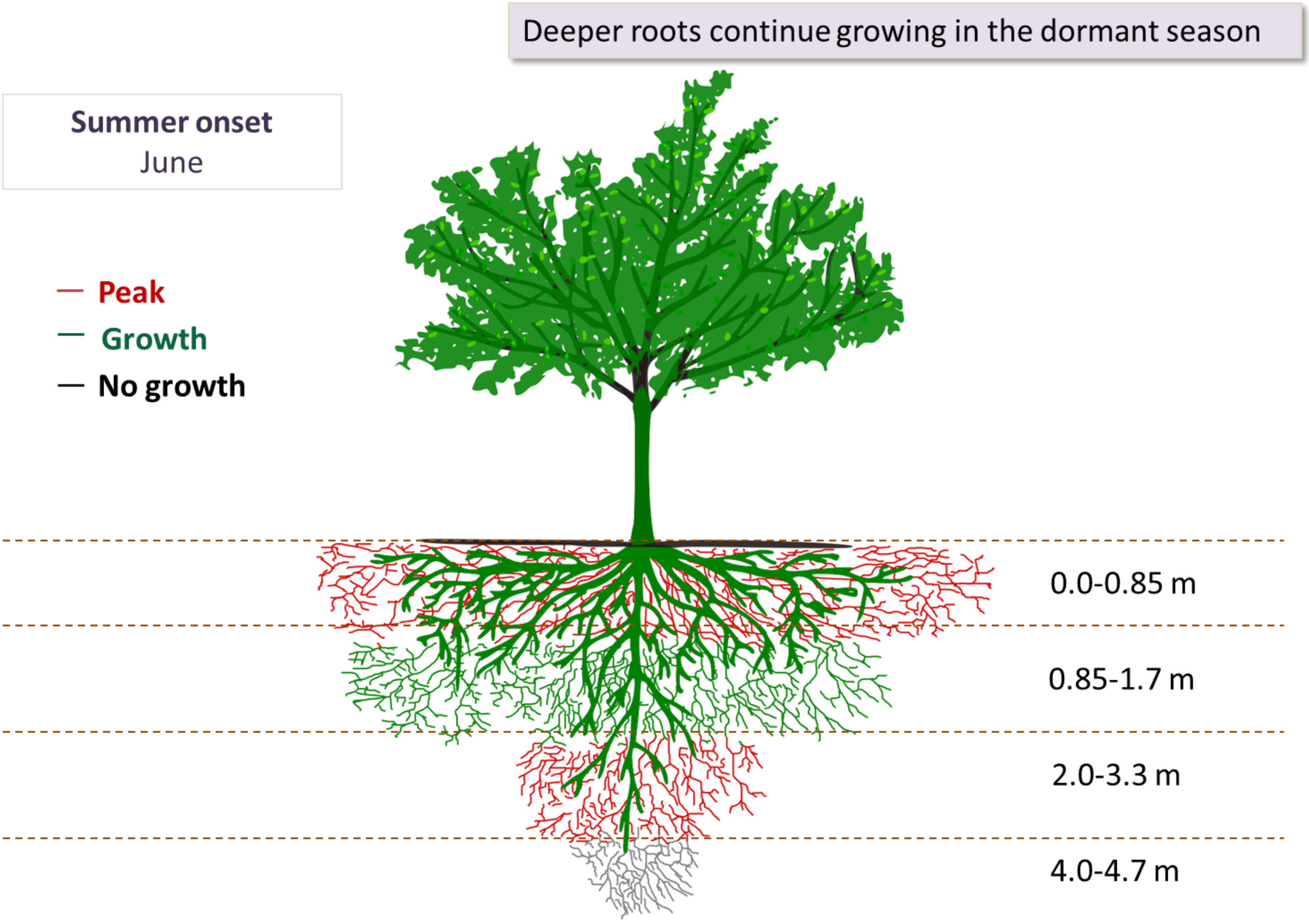
Deeper roots continue growing in the dormant season

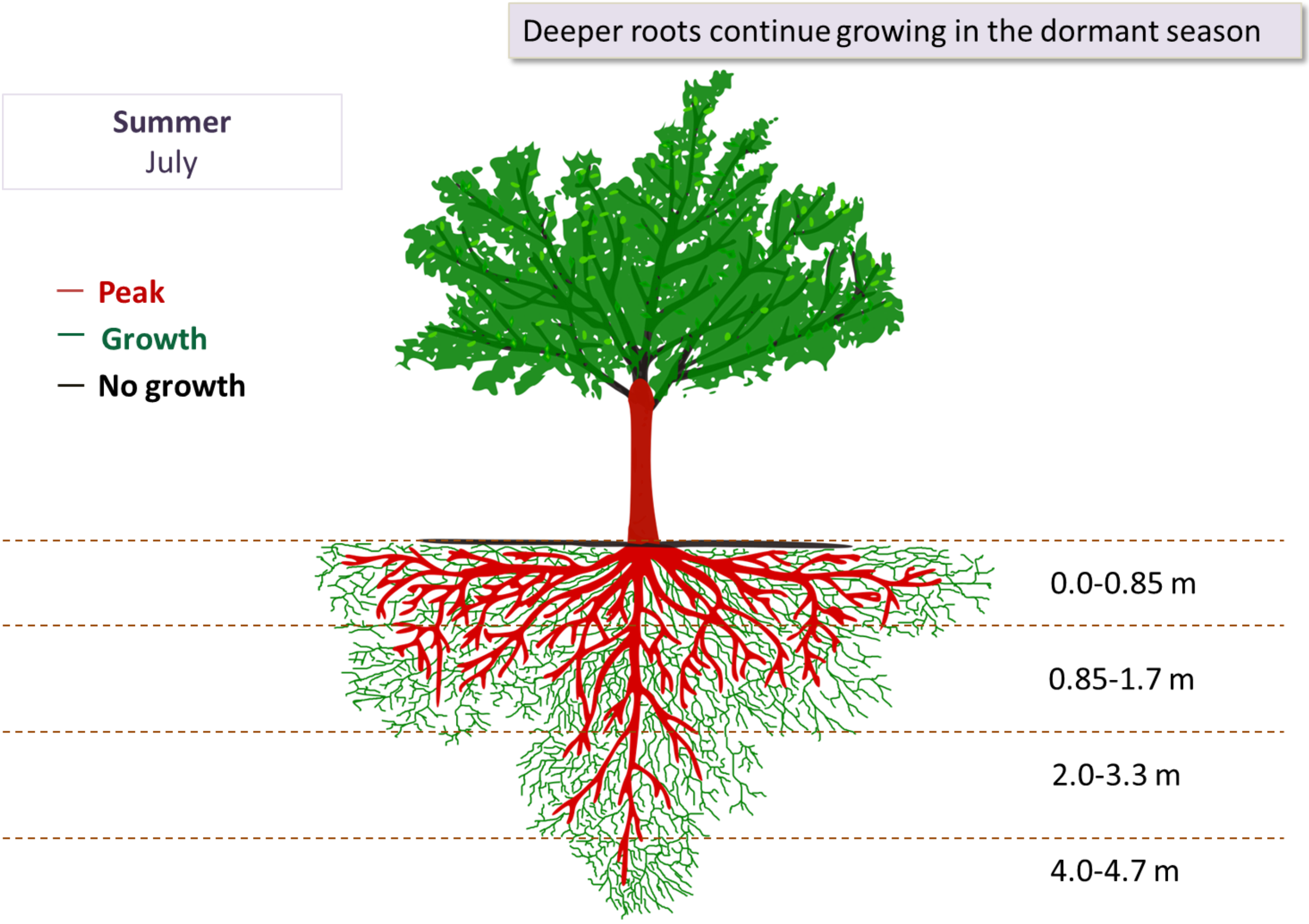


Deeper roots continue growing in the dormant season

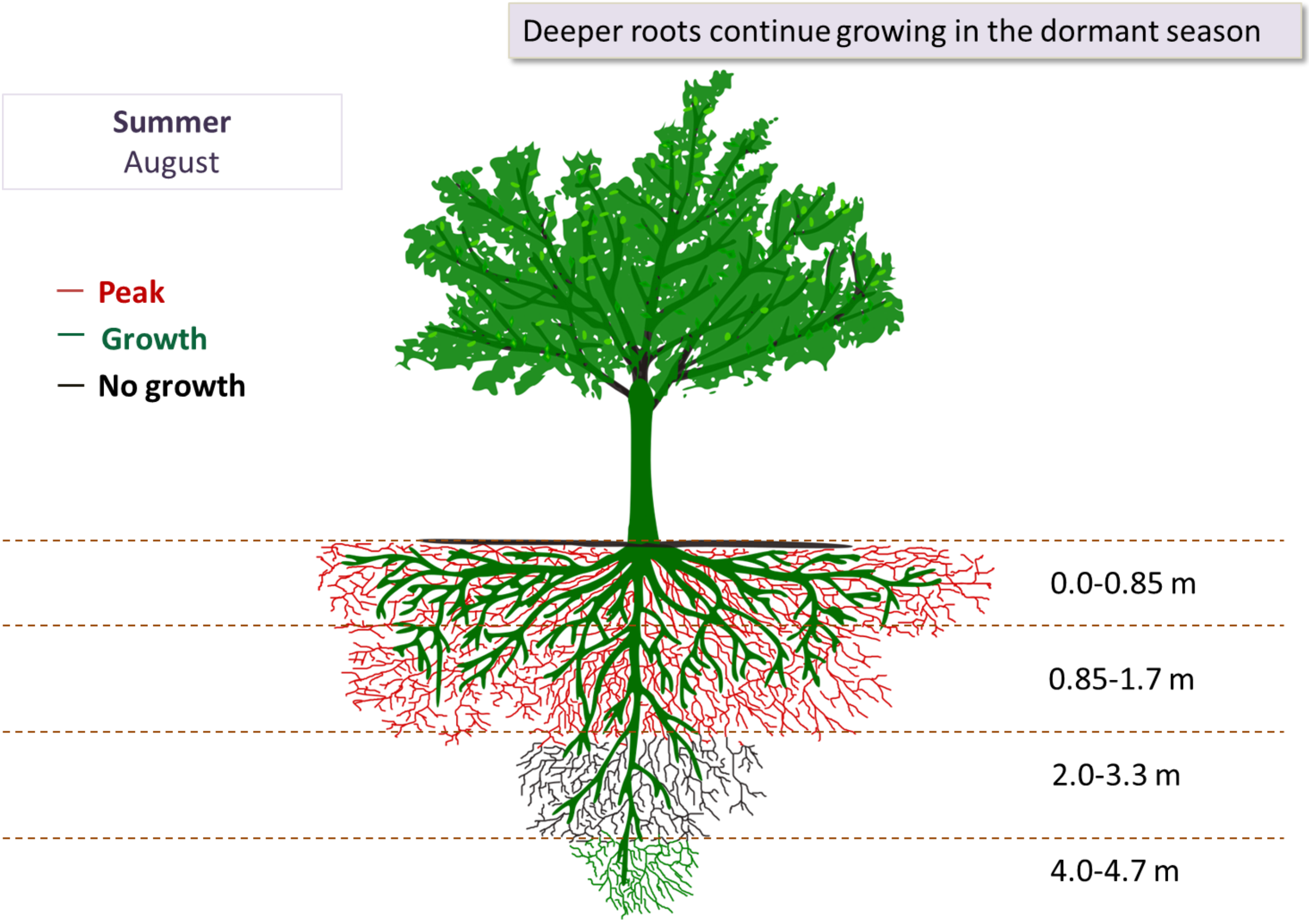
Spring
May




Deeper roots continue growing in the dormant season
Summer onset
June
— Peak
— Growth
— No growth
0.0-0.85 m
0.85-1.7 m
2.0-3.3 m
4.0-4.7 m

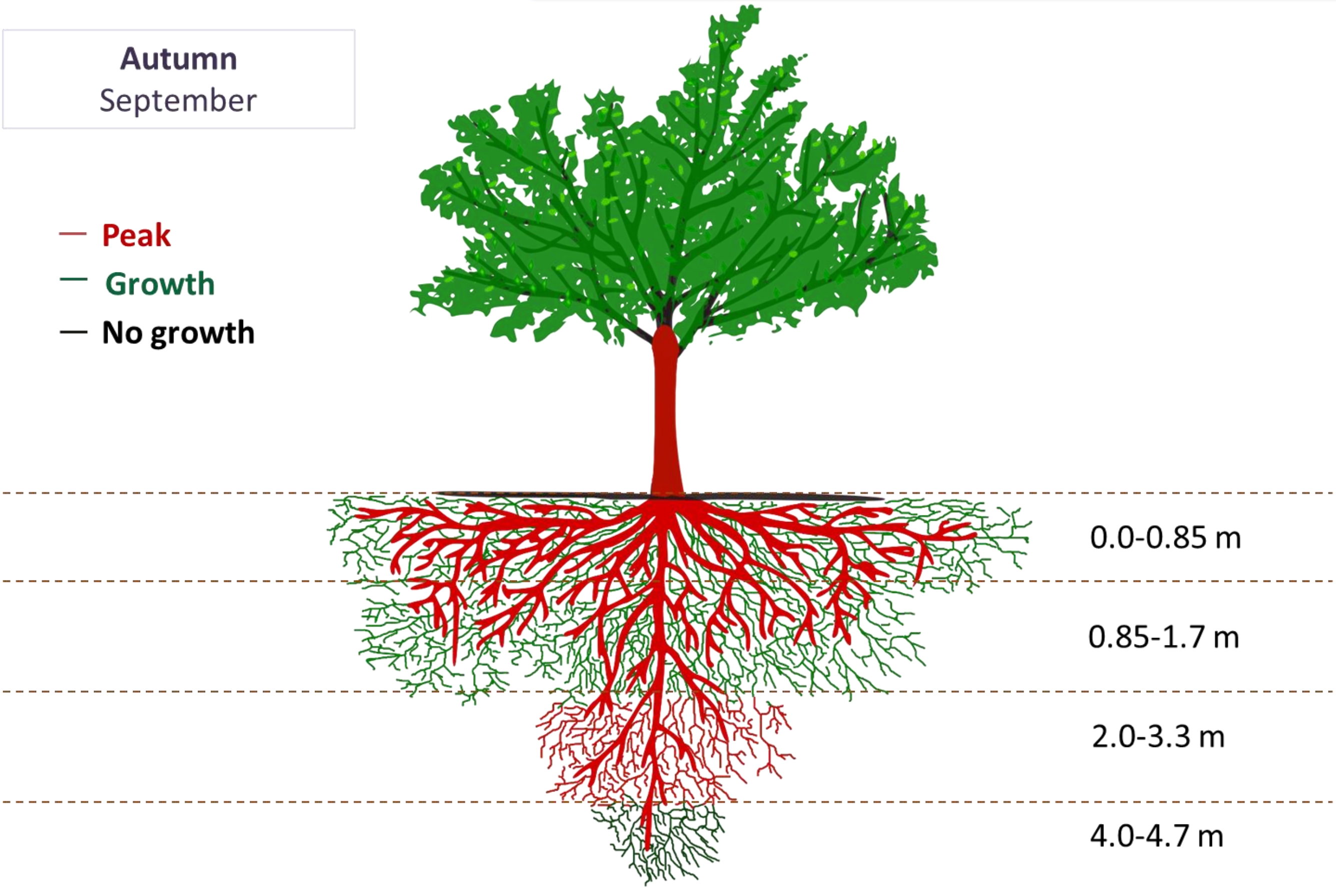


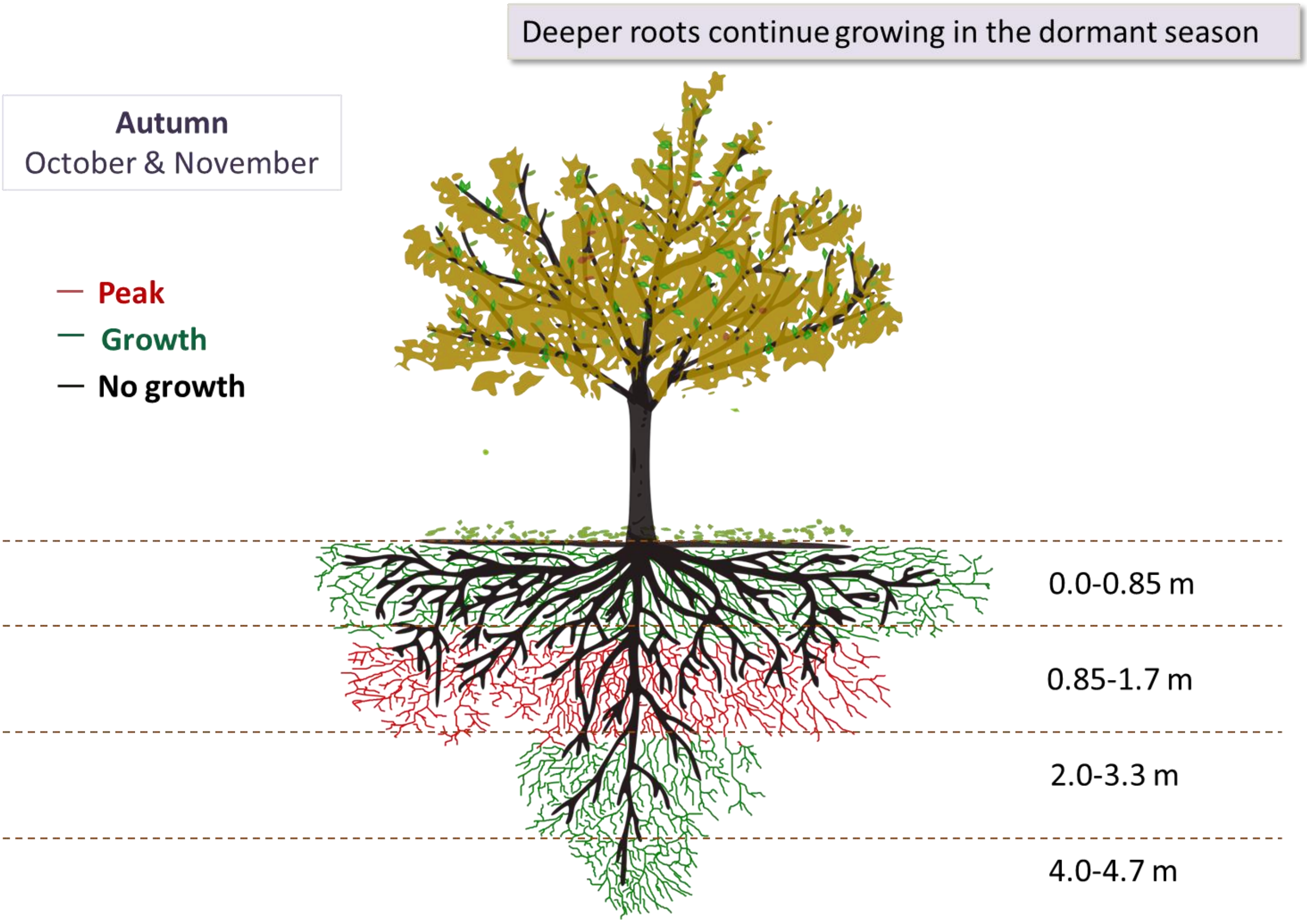
Deeper roots continue growing in the dormant season


Deeper roots continue growing in the dormant season
Summer
August
— Peak
— Growth
— No growth
0.0-0.85 m
0.85-1.7 m
2.0-3.3 m
4.0-4.7 m

Deeper roots continue growing in the dormant season

Autumn
September




Deeper roots continue growing in the dormant season
Autumn
October & November
Peak
Growth
No growth
0.0-0.85 m
0.85-1.7 m
2.0-3.3 m
4.0-4.7 m

Deeper roots continue growing in the dormant season